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**HIGH-AFFINITY LIGANDS OF  
BASIC FIBROBLAST GROWTH FACTOR AND THROMBIN**

**FIELD OF THE INVENTION**

Described herein are methods for identifying and preparing high-affinity nucleic acid ligands to basic fibroblast growth factor (bFGF) and thrombin. The method utilized herein for identifying such ligands is called SELEX, an acronym for Systematic Evolution of Ligands by Exponential Enrichment. Included within the scope of this invention are the specific ligands identified pursuant to such methods. Specifically, nucleic acid ligands are described to bFGF and thrombin. Also, included within the scope of this invention are modified nucleic acid ligands to bFGF and thrombin. Further included are mimetic nucleic acid ligands that are informed by the nucleic acid ligands identified herein. Specifically, disclosed are 2'-amino (2'-NH<sub>2</sub>) modified RNA ligands to bFGF. 2'-NH<sub>2</sub>-modified RNA ligands to bFGF were identified which inhibited the biological activity of bFGF both *in vivo* and *in vitro*. Further included in this invention are single stranded DNA ligands to thrombin and bFGF.

**BACKGROUND OF THE INVENTION**

Most proteins or small molecules are not known to specifically bind to nucleic acids. The known protein exceptions are those regulatory proteins such as repressors, polymerases, activators and tie-like which function in a living cell to bring about the transfer of genetic information encoded in the nucleic acids into cellular structures and the replication of the genetic material. Furthermore, small molecules such as GMP bind to some intron RNAs.

Living matter has evolved to limit the function of nucleic acids to a largely informational role. The central dogma, as postulated by Crick, both originally and in expanded form, proposes that nucleic acids (either RNA or DNA) can serve as templates for

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the synthesis of other nucleic acids through replicative processes that "read" the information in a template nucleic acid and thus yield complementary nucleic acids. All of the experimental paradigms for genetics and gene expression depend on these properties of nucleic acids; in essence, double-stranded nucleic acids are informationally redundant because of the chemical concept of base pairs and because replicative processes are able to use that base pairing in a relatively error-free manner.

The individual components of proteins, the twenty natural amino acids, possess sufficient chemical differences and activities to provide an enormous breadth of activities for both binding and catalysis. Nucleic acids, however, have been thought to have narrower chemical possibilities than proteins, but to have an informational role that allows genetic information to be passed from virus to virus, cell to cell, and organism to organism. In this context nucleic acid components, the nucleotides, possess only pairs of surfaces that allow informational redundancy within a Watson-Crick base pair. Nucleic acid components need not possess chemical differences and activities sufficient for either a wide range of binding or catalysis.

However, some nucleic acids found in nature do participate in binding to certain target molecules and even a few instances of catalysis have been reported. The range of activities of this kind is narrow compared to proteins and more specifically antibodies. For example, where nucleic acids are known to bind to some protein targets with high affinity and specificity, the binding depends on the exact sequences of nucleotides that comprise the DNA or RNA ligand. Thus, short double-stranded DNA sequences are known to bind to target proteins that repress or activate transcription in both prokaryotes and eukaryotes.

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Other short double-stranded DNA sequences are known to bind to restriction endonucleases, protein targets that can be selected with high affinity and specificity. Other short DNA sequences serve as centromeres and telomeres on chromosomes, presumably by creating ligands for the binding of specific proteins that participate in chromosome mechanics. Thus, double-stranded DNA has a well-known capacity to bind within the nooks and crannies of target proteins whose functions are directed to DNA binding. Single-stranded DNA can also bind to some proteins with high affinity and specificity, although the number of examples is smaller. From the known examples of double-stranded DNA binding proteins, it has become possible to describe some of the binding interactions as involving various protein motifs projecting amino acid side chains into the major groove of B form double-stranded DNA, providing the sequence inspection that allows specificity.

Double-stranded RNA occasionally serves as a ligand for certain proteins, for example, the endonuclease RNase III from *B. coli*. There are more known instances of target proteins that bind to single-stranded RNA ligands, although in these cases the single-stranded RNA often forms a complex three-dimensional shape that includes local regions of intramolecular double-strandedness. The amino-acyl tRNA synthetases bind tightly to tRNA molecules with high specificity. A short region within the genomes of RNA viruses binds tightly and with high specificity to the viral coat proteins. A short sequence of RNA binds to the bacteriophage T4-encoded DNA polymerase, again with high affinity and specificity. Thus, it is possible to find RNA and DNA ligands, either double- or single-stranded, serving as binding partners for specific protein targets. Most known DNA binding proteins bind specifically to double-stranded DNA,

while most RNA binding proteins recognize single-stranded RNA. This statistical bias in the literature no doubt reflects the present biosphere's statistical predisposition to use DNA as a double-stranded genome and RNA as a single-stranded entity in the roles RNA plays beyond serving as a genome. Chemically there is no strong reason to dismiss single-stranded DNA as a fully able partner for specific protein interactions. RNA and DNA have also been found to bind to various antibiotics, such as actinomycin D. A specific single-stranded RNA binds to the antibiotic thiostreptone; specific RNA sequences and structures probably bind to certain other antibiotics, especially those whose function is to inactivate ribosomes in a target organism. A family of evolutionary related RNAs binds with specificity and decent affinity to nucleotides and nucleosides (Bass, B. and Cech, T. (1984) *Nature* 312:820-826), as well as, to one of the twenty amino acids (Yarus, M. (1988) *Science* 240:1751-1758). Catalytic RNAs are now known as well, although these molecules perform over a narrow range of chemical possibilities, which are thus far related largely to phosphodiester transfer reactions and hydrolysis of nucleic acids.

Despite these known instances, the great majority of proteins and other cellular components are thought not to bind to nucleic acids under physiological conditions and such binding as may be observed is non-specific. Either the capacity of nucleic acids to bind other compounds is limited to the relatively few instances enumerated *supra*, or the chemical repertoire of the nucleic acids for specific binding is avoided (selected against) in the structures that occur naturally. The present invention is premised on the inventors' fundamental insight that nucleic acids as chemical compounds can form a

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virtually limitless array of shapes, sizes and configurations, and are capable of a far broader repertoire of binding and catalytic functions than those displayed in biological systems.

The chemical interactions have been explored in cases of certain known instances of protein-nucleic acid binding. For example, the size and sequence of the RNA site of bacteriophage R17 coat protein binding has been identified by Uhlenbeck. (Uhlenbeck et al. (1983) *J. Biomol. Structure Dynamics* 1:539 and Romanuk et al. (1987) *Biochemistry* 26:1563) and coworkers. The minimal natural RNA binding site (21 bases long) for the R17 coat protein was determined by subjecting variable-sized labeled fragments of the mRNA to nitrocellulose filter binding assays in which protein-RNA fragment complexes remain bound to the filter. (Carey et al. (1983) *Biochemistry* 22:601). A number of sequence variants of the minimal R17 coat protein binding site were created *in vitro* in order to determine the contributions of individual nucleic acids to protein binding. It was found that the maintenance of the hairpin loop structure of the binding site was essential for protein binding but, in addition, that nucleotide substitutions at most of the single-stranded residues in the binding site, including a bulged nucleotide in the hairpin stem, significantly affected binding. In similar studies, the binding of bacteriophage Q<sub>B</sub> coat protein to its translational operator was examined (Witherell and Uhlenbeck (1989) *Biochemistry* 28:71). The Q<sub>B</sub> coat protein RNA binding site was found to be similar to that of R17 in size, and in predicted secondary structure, in that it comprised about 20 bases with an 8 base pair hairpin structure which included a bulged nucleotide and a 3 base loop. In contrast to the R17 coat protein binding site, only one of the single-stranded residues of the loop is essential for binding and the presence of the

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bulged nucleotide is not required. The protein-RNA binding interactions involved in translational regulation display significant specificity.

Nucleic acids are known to form secondary and tertiary structures in solution. The double-stranded forms of DNA include the so-called B double-helical form, Z-DNA and superhelical twists (Rich, A. et al. (1984) *Ann. Rev. Biochem.* 53:791-846). Single-stranded RNA forms localized regions of secondary structure such as hairpin loops and pseudoknot structures (Schimmel, P. (1989) *Cell* 58:9-12). However, little is known concerning the effects of unpaired loop nucleotides on stability of loop structure, kinetics of formation and denaturation, thermodynamics, and almost nothing is known of tertiary structures and three dimensional shape, nor of the kinetics and thermodynamics of tertiary folding in nucleic acids (Tuerk, C. et al. (1988) *Proc. Natl. Acad. Sci. USA* 85:1364-1368).

A type of *in vitro* evolution was reported in replication of the RNA bacteriophage Q<sub>B</sub>. (Mills, D.R. et al. (1967) *Proc. Natl. Acad. Sci. USA* 54:217-224; Levinsohn, R. and Spiegelman, S. (1968) *Proc. Natl. Acad. Sci. USA* 55:866-872; Levinsohn, R. and Spiegelman, S. (1969) *Proc. Natl. Acad. Sci. USA* 56:805-811; Saffhill, R. et al. (1970) *J. Mol. Biol.* 51:531-539; Kacian, D.L. et al. (1972) *Proc. Natl. Acad. Sci. USA* 69:3038-3042; Mills, D.R. et al. (1973) *Science* 180:916-927). The phage RNA serves as a poly-cistrionic messenger RNA directing translation of phage-specific proteins and also as a template for its own replication catalyzed by Q<sub>B</sub> RNA replicase. This RNA replicase was shown to be highly specific for its own RNA template. During the course of cycles of replication *in vitro* small variant RNAs were isolated which were also replicated by Q<sub>B</sub> replicase. Minor alterations in the conditions under which cycles of replication were performed were found to result in the accumulation of

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different RNAs, presumably because their replication was favored under the altered conditions. In these experiments, the selected RNA had to be bound efficiently by the replicase to initiate replication and had to serve as a kinetically favored template during elongation of RNA. Kramer et al. (1974) J. Mol.

Biol. 89:719 reported the isolation of a mutant RNA template of Qβ replicase, the replication of which was more resistant to inhibition by ethidium bromide than

the natural template. It was suggested that this mutant was not present in the initial RNA population,

but was generated by sequential mutation during cycles of in vitro replication with Qβ replicase. The only source of variation during selection was the intrinsic error rate during elongation by Qβ replicase. In these

studies what was termed "selection" occurred by preferential amplification of one or more of a limited number of spontaneous variants of an initially homogenous RNA sequence. There was no selection of a desired result, only that which was intrinsic to the mode of action of Qβ replicase.

Joyce and Robertson (Joyce (1989) in RNA: Catalysis, Splicing, Evolution, Belfort and Shub (eds.), Elsevier, Amsterdam pp. 83-87; and Robertson and Joyce (1990) Nature 344:467-468) reported a method for identifying RNAs which specifically cleave single-stranded DNA. The selection for catalytic activity was based on the ability of the ribozyme to catalyze the cleavage of a substrate RNA or DNA at a specific position and transfer the 3'-end of the substrate to the 3'-end of the ribozyme. The product of the desired reaction was selected by using a deoxyoligonucleotide primer which could bind only to the completed product across the junction formed by the catalytic reaction and allowed selective reverse transcription of the ribozyme sequence. The selected catalytic sequences were amplified by attachment of the promoter of T7 RNA

polymerase to the 3'-end of the cDNA, followed by transcription to RNA. The method was employed to identify from a small number of ribozyme variants the variant that was most reactive for cleavage of a selected substrate.

The prior art has taught or suggested only a limited range of chemical functions for nucleic acids in their interactions with other substances, namely, as targets for proteins that have evolved to bind certain specific oligonucleotide sequences; and more recently, as catalysts with a limited range of activities. Prior "selection" experiments have been limited to a narrow range of variants of a previously described function.

U.S. Patent Application Serial No. 07/536,428, filed June 11, 1990, entitled Systematic Evolution of Ligands by Exponential Enrichment, now abandoned, U.S. Patent No. 5,270,163, issued December 14, 1993, and U.S. Patent Application Serial Number 07/714,331, filed June 10, 1991, both entitled Nucleic Acid Ligands (See also PCT/IS91/04078) describe a fundamentally novel method for identifying a nucleic acid ligand for any desired target. Each of these applications, collectively referred to herein as the SELEX Patent Applications, is specifically incorporated herein by reference.

The method of the SELEX Patent Applications is based on the unique insight that nucleic acids have sufficient capacity for forming a variety of two- and three-dimensional structures and sufficient chemical versatility available within their monomers to act as ligands (form specific binding pairs) with virtually any chemical compound, whether large or small in size. The method involves selection from a mixture of candidates and step-wise iterations of structural improvement, using the same general selection theme, to achieve virtually any desired criterion of binding affinity and selectivity. Starting from a mixture of

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nucleic acids, preferably comprising a segment of randomized sequence, the method, termed SELEX herein, includes steps of contacting the mixture with the target under conditions favorable for binding, partitioning unbound nucleic acids from those nucleic acids which have bound to target molecules,

dissociating the nucleic acid-target pairs, amplifying the nucleic acids dissociated from the nucleic acid-

target pairs to yield a ligand-enriched mixture of nucleic acids, then reiterating the steps of binding,

partitioning, dissociating and amplifying through as many cycles as desired.

While not bound by theory, SELEX is based on

the inventors' insight that within a nucleic acid

mixture containing a large number of possible sequences and structures there is a wide range of binding

affinities for a given target. A nucleic acid mixture comprising, for example, a 20 nucleotide randomized segment can have 4<sup>20</sup> candidate possibilities. Those

which have the higher affinity constants for the target

are most likely to bind to the target. After

partitioning, dissociation and amplification, a second

nucleic acid mixture is generated, enriched for the

higher binding affinity candidates. Additional rounds

of selection progressively favor the best ligands until

the resulting nucleic acid mixture is predominantly

composed of only one or a few sequences. These can

then be cloned, sequenced and individually tested for

binding affinity as pure ligands.

Cycles of selection and amplification are

repeated until a desired goal is achieved. In the most

general case, selection/amplification is continued

until no significant improvement in binding strength is

achieved on repetition of the cycle. The method may be

used to sample as many as about 10<sup>11</sup> different nucleic

acid species. The nucleic acids of the test mixture

preferably include a randomized sequence portion as

well as conserved sequences necessary for efficient amplification. Nucleic acid sequence variants can be produced in a number of ways including synthesis of randomized nucleic acid sequences and size selection from randomly cleaved cellular nucleic acids. The variable sequence portion may contain fully or partially random sequence; it may also contain subportions of conserved sequence incorporated with randomized sequence. Sequence variation in test nucleic acids can be introduced or increased by mutagenesis before or during the

selection/amplification iterations.

In one embodiment of the method of the SELEX Patent Applications, the selection process is so efficient at isolating those nucleic acid ligands that bind most strongly to the selected target, that only one cycle of selection and amplification is required. Such an efficient selection may occur, for example, in a chromatographic-type process wherein the ability of nucleic acids to associate with targets bound on a column operates in such a manner that the column is sufficiently able to allow separation and isolation of the highest affinity nucleic acid ligands.

In many cases, it is not necessarily desirable to perform the iterative steps of SELEX until a single nucleic acid ligand is identified. The target-specific nucleic acid ligand solution may include a family of nucleic acid structures or motifs that have a number of conserved sequences and a number of sequences which can be substituted or added without significantly effecting the affinity of the nucleic acid ligands to the target. By terminating the SELEX process prior to completion, it is possible to determine the sequence of a number of members of the nucleic acid ligand solution family.

A variety of nucleic acid primary, secondary and tertiary structures are known to exist. The

- 5 nucleic acids, preferably comprising a segment of randomized sequence, the method, termed SELEX herein, includes steps of contacting the mixture with the target under conditions favorable for binding, partitioning unbound nucleic acids from those nucleic acids which have bound to target molecules,
- 10 dissociating the nucleic acid-target pairs, amplifying the nucleic acids dissociated from the nucleic acid-target pairs to yield a ligand-enriched mixture of nucleic acids, then reiterating the steps of binding, partitioning, dissociating and amplifying through as many cycles as desired.
- 15 While not bound by theory, SELEX is based on the inventors' insight that within a nucleic acid mixture containing a large number of possible sequences and structures there is a wide range of binding affinities for a given target. A nucleic acid mixture comprising, for example, a 20 nucleotide randomized segment can have 4<sup>20</sup> candidate possibilities. Those which have the higher affinity constants for the target are most likely to bind to the target. After partitioning, dissociation and amplification, a second nucleic acid mixture is generated, enriched for the higher binding affinity candidates. Additional rounds of selection progressively favor the best ligands until the resulting nucleic acid mixture is predominantly composed of only one or a few sequences. These can then be cloned, sequenced and individually tested for binding affinity as pure ligands.
- 20 Cycles of selection and amplification are repeated until a desired goal is achieved. In the most general case, selection/amplification is continued until no significant improvement in binding strength is achieved on repetition of the cycle. The method may be used to sample as many as about 10<sup>11</sup> different nucleic acid species. The nucleic acids of the test mixture preferably include a randomized sequence portion as
- 25 well as conserved sequences necessary for efficient amplification. Nucleic acid sequence variants can be produced in a number of ways including synthesis of randomized nucleic acid sequences and size selection from randomly cleaved cellular nucleic acids. The variable sequence portion may contain fully or partially random sequence; it may also contain subportions of conserved sequence incorporated with randomized sequence. Sequence variation in test nucleic acids can be introduced or increased by mutagenesis before or during the selection/amplification iterations.
- 30 In one embodiment of the method of the SELEX Patent Applications, the selection process is so efficient at isolating those nucleic acid ligands that bind most strongly to the selected target, that only one cycle of selection and amplification is required. Such an efficient selection may occur, for example, in a chromatographic-type process wherein the ability of nucleic acids to associate with targets bound on a column operates in such a manner that the column is sufficiently able to allow separation and isolation of the highest affinity nucleic acid ligands.
- 35 In many cases, it is not necessarily desirable to perform the iterative steps of SELEX until a single nucleic acid ligand is identified. The target-specific nucleic acid ligand solution may include a family of nucleic acid structures or motifs that have a number of conserved sequences and a number of sequences which can be substituted or added without significantly effecting the affinity of the nucleic acid ligands to the target. By terminating the SELEX process prior to completion, it is possible to determine the sequence of a number of members of the nucleic acid ligand solution family.

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structures or motifs that have been shown most commonly

to be involved in non-Watson-Crick type interactions

are referred to as hairpin loops, symmetric and

asymmetric bulges, pseudoknots and myriad combinations

of the same. Almost all known cases of such motifs

suggest that they can be formed in a nucleic acid

sequence of no more than 30 nucleotides. For this

reason, it is often preferred that SELEX procedures

with contiguous randomized segments be initiated with

nucleic acid sequences containing a randomized segment

of between about 20-50 nucleotides.

The SELEX Patent Applications also describe

methods for obtaining nucleic acid ligands that bind to

more than one site on the target molecule, and to

nucleic acid ligands that include non-nucleic acid

species that bind to specific sites on the target. The

SELEX method provides means for isolating and

identifying nucleic acid ligands which bind to any

envisionable target. However, in preferred embodiments

the SELEX method is applied to situations where the

target is a protein, including both nucleic acid-

binding proteins and proteins not known to bind nucleic

acids as part of their biological function.

Basic fibroblast growth factor (bFGF) is a

multifunctional effector for many cells of mesenchymal

and neuroectodermal origin (Rifkin & Moscatelli (1989)

J. Cell Biol. 109:1; Baird & Bohlen (1991) in *Peptide*

*Growth Factors and Their Receptors* (Sporn, M. B. &

Roberts, A. B., eds.), pp. 369-18, Springer, N.Y.;

Basilico & Moscatelli (1992) Adv. Cancer Res. 59:115).

It is one of the most studied and best characterized

members of a family of related proteins that also

includes acidic FGF (Jaye et al. (1986) Science

233:541; Abraham et al. (1986) Science 233:545), int-2

(Moore et al. (1986) EMBO J. 5:919), KGF/Hst/KS3

(Delli Bovi et al. (1987) Cell 50:729; Taira et al. (1987) Proc. Natl. Acad. Sci. USA 84:2980), FGF-5 (Zhan

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et al. (1988) Mol. Cell. Biol. 8:3487), FGF-6 (Marics et al. (1988) Oncogene 4:335) and keratinocyte growth factor/FGF-7 (Ginch et al. (1989) Science 245:752).

In vitro, bFGF stimulates cell proliferation, migration and induction of plasminogen activator and collagenase activities (Presta et al. (1986) Mol. Cell. Biol. 5:4060; Moscatelli et al. (1989) J. Cell Biol. 108:671). In vivo, it is one of the most potent inducers of neovascularization. Its angiogenic

activity in vivo suggests a role in tissue remodeling and wound healing, but also, in some disease states

that are characterized by pathological neovascularization such as tumor proliferation, tumor

metastasis, diabetic retinopathy and rheumatoid

arthritis (Polkman & Klagsbrun (1987) Science 235:442; Gospodarowicz (1991) Cell Biology Review 25:307).

Although bFGF does not have a signal sequence for secretion, it is found on both sides of the plasma membrane, presumably being exported via exocytosis (Vlodavsky et al. (1991) Trends Biol. Sci. 16:268; Mignatti & Rifkin (1991) J. Cell. Biochem. 47:61). In the extracellular matrix, it is typically associated

with a fraction that contains heparan sulfate proteoglycans. Indeed, heparin affinity chromatography

has been a useful method for purification of this and other heparin-binding growth factors. Heparin is a glycosaminoglycan composed of chains of alternating

residues of D-glucosamine and uronic acid. In cell

culture, bFGF binds to low- and high-affinity sites.

The low-affinity sites are composed of cell-associated heparan sulfate proteoglycans to which bFGF binds with

approximately nanomolar affinity (Moscatelli (1987) J. Cell. Physiol. 131:123). All biological effects of

bFGF are mediated through interaction with the high-affinity binding sites (10-100 pM) that represent the

dimeric tyrosine kinase FGF receptor (Deno et al.

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(1992) *J. Biol. Chem.* **267**:1470).

Five bFGF receptor genes have been identified to date, each of which can produce several structural variants as a result of alternative mRNA splicing (Armstrong et al. (1992) *Cancer Res.* **52**:2004; Ueno et al. (1992) *J. Biol. Chem.* **267**:1470). There is substantial evidence that the low- and the high-affinity binding sites act cooperatively in determining the overall affinity of bFGF. Experiments with mutant cell lines that are deficient in glycosaminoglycan synthesis (Fayon et al. (1991) *Cell* **64**:81) or heparitinase treated cells (Reproger et al. (1991) *Science* **252**:1705) have shown that binding of either cell-associated heparan sulfate or, in its absence, exogenously added heparin to bFGF is required for signaling via the tyrosine kinase receptor. Recent resolution of observed Kd into its kinetic components demonstrates that while the association rates of bFGF to the low- and the high-affinity sites are comparable, the dissociation rate of bFGF from the cell surface receptor is 23-fold slower than that for the cell-associated heparan sulfate (Nugent & Edelman (1992) *Biochemistry* **31**:8976). The slower off-rate, however, is only observed when the receptor is bound to the cell surface suggesting that simultaneous binding to both sites contributes to the overall high-affinity binding. This is plausible in light of the observation that the heparin-binding and the receptor-binding sites are located on adjacent, but separate regions of the molecule, as determined from the recently solved X-ray crystal structure of bFGF (Zhang et al. (1991) *Proc. Natl. Acad. Sci. USA* **88**:3445; Eriksson et al. (1991) *Proc. Natl. Acad. Sci. USA* **88**:3441). In addition to having a number of undesirable side effects and substantial toxicity, suramin is known to interact with several other heparin-binding growth factors which makes linking of its beneficial therapeutic effects to specific drug-protein interactions difficult (La Rocca et al. (1990) *Cancer Cells* **2**:06). Anti-angiogenic properties of certain heparin preparations have also been observed (Folkman et al. (1983) *Science* **221**:719; Crum et al. (1985) *Science* **230**:375) and these effects are probably based at least in part on their ability to interfere with bFGF signaling. While the specific heparin fraction that contributes to bFGF binding is now partially elucidated (Ishai-Michaeli et al. (1992) *Biochemistry* **31**:2060; Turnbull et al. (1992) *J. Biol. Chem.* **267**:1037), a typical heparin preparation is heterogeneous with respect to size, degree of sulfation

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bFGF is now known to play a key role in the development of smooth-muscle cell lesions following vascular injury (Reidy et al. (1992) *Circulation, Suppl.* III **86**:III-43). Overexpression of bFGF (and other members of the FGF family) is correlated with many malignant disorders (Halahan et al. (1991) *Ann. N. Y. Acad. Sci.* **638**:232; Takahashi et al. (1990) *Proc. Natl. Acad. Sci. USA* **87**:5710; Fujimoto et al. (1991) *Biochem. Biophys. Res. Commun.* **180**:386) and recently, neutralizing anti-bFGF antibodies have been found to suppress solid tumor growth *in vivo* by inhibiting tumor-linked angiogenesis (Hori et al. (1991) *Cancer Res.* **51**:6180). Notable in this regard is the recent therapeutic examination of suramin, a polysulfated naphthalene derivative with known antiprotozoal activity, as an anti-tumor agent. Suramin is believed to inhibit the activity of bFGF through binding in the polyanion binding site and disrupting interaction of the growth factor with its receptor (Middaugh et al. (1992) *Biochemistry* **31**:916; Eriksson et al. (1991) *Proc. Natl. Acad. Sci. USA* **88**:3441). In addition to having a number of

15 suramin is known to interact with several other heparin-binding growth factors which makes linking of its beneficial therapeutic effects to specific drug-protein interactions difficult (La Rocca et al. (1990) *Cancer Cells* **2**:06). Anti-angiogenic properties of certain heparin preparations have also been observed (Folkman et al. (1983) *Science* **221**:719; Crum et al. (1985) *Science* **230**:375) and these effects are probably based at least in part on their ability to interfere with bFGF signaling. While the specific heparin fraction that contributes to bFGF binding is now partially elucidated (Ishai-Michaeli et al. (1992) *Biochemistry* **31**:2060; Turnbull et al. (1992) *J. Biol. Chem.* **267**:1037), a typical heparin preparation is heterogeneous with respect to size, degree of sulfation

5 (Armstrong et al. (1992) *Cancer Res.* **52**:2004; Ueno et al. (1992) *J. Biol. Chem.* **267**:1470).

10 Gospodarowicz (1991) *Cell Biology Reviews* **25**:307).

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and iduronic acid content. Additionally, heparin also affects many enzymes and growth factors. Excluding monoclonal antibodies, therefore, specific antagonists of bFGF are not known.

Thrombin is a multifunctional serine protease that has important procoagulant and anticoagulant activities. As a procoagulant enzyme thrombin clots fibrinogen, activates clotting factors V, VIII, and XII, and activates platelets. The specific cleavage of fibrinogen by thrombin initiates the polymerization of fibrin monomers, a primary event in blood clot formation. The central event in the formation of platelet thrombi is the activation of platelets from the "nonbinding" to the "binding" mode and thrombin is the most potent physiologic activator of platelet aggregation (Berndt and Phillips (1981) in *Platelets in Biology and Pathology*, J. L. Gordon, ed. (Amsterdam:Elsevier/North Holland Biomedical Press), pp. 43-74; Hansen and Harker (1988) *Proc. Natl. Acad. Sci. USA* 85:3184-3188; Eict et al. (1983) *J. Clin. Invest.* 84:18-27). Thus, as a procoagulant, thrombin plays a key role in the arrest of bleeding (physiologic hemostasis) and formation of vasoocclusive thrombi (pathologic thrombosis).

As an anticoagulant thrombin binds to thrombomodulin (TM), a glycoprotein expressed on the surface of vascular endothelial cells. TM alters substrate specificity from fibrinogen and platelets to protein C through a combination of an allosteric change in the active site conformation and an overlap of the TM and fibrinogen binding sites on thrombin. Activated protein C, in the presence of a phospholipid surface,  $\text{Ca}^{2+}$ , and a second vitamin K-dependent protein cofactor, protein S, inhibits coagulation by proteolytically degrading factors Va and VIIa. Thus, the formation of the thrombin-TM complex converts thrombin from a procoagulant to an anticoagulant

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enzyme, and the normal balance between these opposing activities is critical to the regulation of hemostasis.

Thrombin is also involved in biological responses that are far removed from the clotting system (reviewed in Zimmerman et al. (1986) *Ann. N. Y. Acad. Sci.* 485:349-369; Marx (1992) *Science* 256:1278-1280). Thrombin is chemotactic for monocytes (Bar-Shavit et al. (1983) *Science* 220:728-730), mitogenic for lymphocytes (Chen et al. (1976) *Exp. Cell Res.* 101:41-46), mesenchymal cells (Chen and Buchanan (1975) *Proc. Natl. Acad. Sci. USA* 72:131-138), and fibroblasts (Marx (1992) *Science* 256:1278-1280). Thrombin activates endothelial cells to express the neutrophil adhesive protein GMP-140 (PADGEM) (Hattori et al. (1989) *J. Biol. Chem.* 264:7768-7771) and produce platelet-derived growth factor (Daniel et al. (1986) *J. Biol. Chem.* 261:9579-9582). Recently it has been shown that thrombin causes cultured nerve cells to retract their neurites (reviewed in Marx (1992) *Science* 256:1278-1280).

The mechanism by which thrombin activates platelets and endothelial cells is through a functional thrombin receptor found on these cells. A putative thrombin cleavage site (LRR/S) in the receptor suggests that the thrombin receptor is activated by proteolytic cleavage of the receptor. This cleavage event "unmasks" an N-terminal domain which then acts as the ligand, activating the receptor (Vu et al. (1991) *Cell* 64:1057-1068). Vascular injury and thrombus formation represent the key events in the pathogenesis of various vascular diseases, including atherosclerosis. The pathogenic processes of the activation of platelets and/or the clotting system leading to thrombosis in various disease states and in various sites, such as the coronary arteries, cardiac chambers, and prosthetic heart valves, appear to be different. Therefore, the

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use of a platelet inhibitor, an anticoagulant, or a combination of both may be required in conjunction with thrombolytics to open closed vessels and prevent reocclusion.

Controlled proteolysis by compounds of the coagulation cascade is critical for hemostasis. As a result, a variety of complex regulatory systems exist that are based, in part, on a series of highly specific protease inhibitors. In a pathological situation functional inhibitory activity can be interrupted by excessive production of active protease or inactivation of inhibitory activity. Perspiration of inflammation in response to multiple trauma (tissue damage) or infection (sepsis) depends on proteolytic enzymes, both of plasma cascade systems, including thrombin, and lysosomal origin. Multiple organ failure (MOF) in these cases is enhanced by the concurrently arising imbalance between proteases and their inhibitory regulators. An imbalance of thrombin activity in the brain may lead to neurodegenerative diseases.

Thrombin is naturally inhibited in hemostasis by binding to antithrombin III (ATIII), in a heparin-dependent reaction. Heparin exerts its effect through its ability to accelerate the action of ATIII. In the brain, protease nexin (PN-1) may be the natural inhibitor of thrombin to regulate neurite outgrowth.

As stated above, heparin is a glycosaminoglycan composed of chains of alternating residues of D-glucosamine and uronic acid. Its anticoagulant effect is mediated through its interaction with ATIII. When heparin binds ATIII, the conformation of ATIII is altered, and it becomes a significantly enhanced inhibitor of thrombin. Although heparin is generally considered to be effective for certain indications, it is believed that the physical size of the ATIII/heparin complex prevents access to much of the biologically active thrombin in the body,

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thus diminishing its ability to inhibit clot formation. Side effects of heparin include bleeding, thrombocytopenia, osteoporosis, skin necrosis, alp, hypersensitivity and hypoadenosorism.

Hirudin is a potent peptide inhibitor of thrombin derived from the European medicinal leech *Hirudis medicinalis*. Hirudin inhibits all known functions of  $\alpha$ -thrombin, and has been shown to bind thrombin at two separate sites kinetically; a high affinity site at or near the catalytic site for serine protease activity and a second anionic exosite. The anionic exosite also binds fibrinogen, heparin, TM and probably the receptor involved in mediating the activation of platelets and endothelial cells. A C-terminal hirudin peptide -- which has been shown by co-crystallization with thrombin to bind in the anionic exosite -- has inhibitory effects on fibrin formation, platelet and endothelial cell activation, and Protein C activation via TM binding, presumably by competing for binding at this site. This peptide does not inhibit proteolytic activity towards tripeptide chromogenic substrates, Factor V or X.

The structure of thrombin makes it a particularly desirable target for nucleic acid binding, due to the anionic exosite. Site-directed mutagenesis within this site has shown that fibrinogen-clotting and TM binding activities are separable. Conceivably, an RNA ligand could be selected that has procoagulatory and/or anticoagulatory effects depending on how it interacts with thrombin, i.e., which substrate it mimics.

A single stranded DNA ligand to thrombin has been prepared according to a procedure identical to SELEX. See, Bock et al. (1992) *Nature* 355:564-565. A consensus ligand was identified after relatively few rounds of SELEX were performed, that was shown to have some ability to prevent clot formation in vitro. The

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ligand is the 15mer DNA 5'-GGTTGGCTGTCCTGG-3', referred to herein as G15D (SEQ ID No:189). The symmetrical nature of the primary sequence suggests that G15D has a regular fixed tertiary structure. The Kd of G15D to thrombin is about  $2 \times 10^{-7}$ . For effective thrombin inhibition as an anticoagulant, the stronger the affinity of the ligand to thrombin the better.

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The present invention includes methods for identifying and producing nucleic acid ligands and the

Identifying and producing nucleic acid ligands and nucleic acid ligands so identified and produced.

Nucleic acid sequences are provided that are ligands of hepar and thrombin. Specifically, RNA and DNA sequences

are provided that are capable of binding specifically

to bRGP and to thrombin. Included within the invention are the nucleic acid ligand sequences shown in Tables

II-IV (SEQ ID NOS:8-69), Table VIII (SEQ ID NOS:101-

NOS:330-445).  
Also included in this invention are similar

also included in this invention are nucleic acid ligands of bFGF that are inhibitors of bFGF.

Specifically, RNA ligands are identified and described which inhibit the binding of *bFGF* to its receptors.

Further included in this invention is a

method of identifying nucleic acid ligands and ligand sequences to bFGF and thrombin comprising the steps of

a) preparing a candidate mixture of nucleic acids; b)

partitioning between members of said candidate mixture on the basis of affinity to BFGF or thrombin; and c)

amplifying the selected molecules to yield a mixture of nucleic acids enriched for nucleic acid sequences with

a relatively higher affinity for binding to bFGF or

More specifically, the present invention relates to thrombin.

includes the RNA ligands to bFGF and to thrombin

identified according to the above-described method, including those ligands listed in Tables II-IV and Tables XII and XIII. Also included are RNA ligands to bFGF and thrombin that are substantially homologous to any of the given ligands and that have substantially the same ability to bind and inhibit bFGF and thrombin. Further included in this invention are RNA ligands to bFGF and thrombin that have substantially the same structural form as the ligands presented herein and that have substantially the same ability to bind and inhibit bFGF and thrombin.

The present invention also includes modified nucleotide sequences based on the nucleic acid ligand sequences identified herein and mixtures of the same. Specifically included in this invention are RNA ligands, that have been modified at the ribose and/or phosphate and/or base positions to increase the *in vivo* stability of the RNA ligand. Other modifications to RNA ligands are encompassed by this invention, including specific alterations in base sequence, and additions of nucleic acids or non-nucleic acid moieties to the original compound. More specifically, included in this invention are the RNA ligands to bFGF, comprising nucleotides modified at the 2'-amino (2'-NH<sub>2</sub>) position shown in Table VIII. The 2'-NH<sub>2</sub>-modified RNA ligands possess improved *in vivo* stability.

The SELEX method utilizing a single-stranded DNA library of nucleic acids was also performed using bFGF and thrombin as the target. Included within the invention, therefore, are the single-stranded DNA ligands to bFGF shown in Tables XI and XII and to thrombin shown in Tables XV and XVI. Also included in the invention are DNA ligands to thrombin that are substantially homologous to the DNA ligands identified herein and that have substantially the same ability to bind thrombin. Further included in this invention are DNA ligands to thrombin that have substantially the

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same structural form as the DNA ligands presented herein and that have substantially the same ability to bind thrombin.

#### 5 BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows binding curves for bFGF Family 1 ligand 7A (SEQ ID NO:10) (Δ), Family 2 ligand 12A (SEQ ID NO:25) (□), random RNA, SELEX experiment A (+) and random RNA, SELEX experiment B (x). The fraction of RNA bound to nitrocellulose filters is plotted as a function of free protein concentration and data points were fitted to equation 2 as defined in Example 3 below. The following concentrations of RNA were used: < 100 pM for 7A and 12A, and 10 nM for random RNAs. Binding reactions were done at 37 °C in phosphate buffered saline containing 0.01% human serum albumin.

Figure 2 shows the effect of bFGF RNA ligands 5A (SEQ ID NO:9) (○), 7A (SEQ ID NO:10) (Δ), 12A (SEQ ID NO:25) (□), 26A (SEQ ID NO:26) (◇), random RNA, SELEX experiment A (+) and random RNA, SELEX experiment B (x) on binding of <sup>125</sup>I-bFGF to the low-affinity (Figure 2A) and the high-affinity (Figure 2B) cell-surface receptors. Experiments were done essentially as described in Roghani & Moscatelli (1992) J. Biol. Chem. 267:22156.

Figure 3 shows the competitive displacement of <sup>32</sup>P-labeled bFGF RNA ligands 5A (SEQ ID NO:9) (○), 7A (SEQ ID NO:10) (Δ), 12A (SEQ ID NO:25) (□), and 26A (SEQ ID NO:26) (◇) by heparin (average molecular weight 5,000 Da). Percent of total input RNA bound to nitrocellulose filters is plotted as a function of heparin concentration. Experiments were done at 37 °C in phosphate buffered saline containing 0.01% human serum albumin, 0.3 μM RNA, and 30 nM bFGF.

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Figure 4 shows the consensus structures for bFGF Family 1 and Family 2 ligands. Y = C or U; R = A or G; W = A or U; H = A, U, or C; D = A, G, or U; N = any base. Complementary bases are primed. Symbols in parenthesis indicate a variable number of bases or base pairs at that position ranging within limits given in the subscript.

Figure 5 shows the binding curves for 2'-NH<sub>2</sub> modified bFGF RNA ligands 21A (SEQ ID NO:104) (○) (SELEX experiment A), 38B (SEQ ID NO:114) (Δ) (SELEX experiment B) and the initial (random) RNAs (A and B) from which these ligands were selected (□, ◇).

Figure 6 shows 2'-NH<sub>2</sub>-modified bFGF RNA ligand inhibition of <sup>125</sup>I-bFGF binding to the low-affinity (Figure 6A) and the high-affinity (Figure 6B) cell surface receptors. The ligands tested were 21A (SEQ ID NO:104) (Δ), 21A-t (SEQ ID NO:186) (○), and random RNA A (□).

Figure 7 shows the possible secondary structures of the 76 nucleotide Class I thrombin RNA clones 6 (SEQ ID NO:211), 15 (SEQ ID NO:212), and 18 (SEQ ID NO:213), and the Class II 72 nucleotide clone 27 (SEQ ID NO:214) as determined from boundary experiments. Boundaries are underlined. The 5' and 3' fixed regions are depicted by small case lettering, the 30N random region by caps and the conserved region by bold caps. The hairpin structures that were synthesized are boxed with the total number of nucleotides indicated.

Figure 8 depicts binding curves for various thrombin ligands. In Figure 8A RNAs with unique 30N sequence motifs (see Table XII) were chosen for binding analysis with human thrombin (Sigma), including the

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three from Class I: RNA 6 (SEQ ID NO:192), RNA 16 (SEQ ID NO:198), and RNA 18 (SEQ ID NO:199), and one from Class II: RNA 27 (SEQ ID NO:209). Binding of bulk RNA sequences of the 30N3 candidate mixture is also shown. In Figure 8B, binding of class I RNA clones 6, 16, 18 and Class II RNA clone 27 is shown, but with human thrombin from Enzyme Research Laboratories. In Figure 8C, binding of the 15mer ssDNA 5'-GGTTGGTGGTGG-3' (G15D) (SEQ ID NO:189), the Class I clone 16 hairpin structures (24R, 39D) (SEQ ID NO:212) and the Class II clone 27 hairpin structure (33R) (SEQ ID NO:214) (see Figure 7 and Table XIII) are shown under identical conditions as in Figure 8B. In the case of the RNA hairpin structures, R denotes RNA synthesis and D denotes transcription from a DNA template.

Figure 9 depicts a binding comparison of thrombin RNA ligands between unmodified RNA and RNA with pyrimidines modified to contain the 2'-NH<sub>2</sub> ribose nucleotide. Figure 9A depicts the binding comparison of bulk RNA 30N candidate mixture and 2'-NH<sub>2</sub> modified 30N candidate mixture. Figure 9B depicts the binding comparison of Class I RNA 16 (SEQ ID NO:198) and 2'-NH<sub>2</sub> modified RNA 16, and Figure 11C depicts the binding comparison of Class II RNA 27 (SEQ ID NO:209) and 2'-NH<sub>2</sub> modified RNA 27 are shown.

Figure 10 depicts the competition experiments between the 15mer ssDNA G15D (SEQ ID NO:189) and the thrombin RNA hairpin ligands of this invention for binding to human thrombin. In Figure 10A the concentration of the tracer G15D is equal to the concentration of protein at 1 μM. The competitors for binding include G15D itself, the 24 and 39 nucleotide RNA hairpin structures from Class I RNA 16 (SEQ ID NO:212), and the 33 nucleotide RNA hairpin structure from Class II RNA 27 (SEQ ID NO:214) (see Figure 7).

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Binding is expressed as the relative fraction G15D bound, which is the ratio of G15D binding with competitor to G15D binding without competitor. In Figure 10B 33 nucleotide hairpin RNA is the tracer and the concentration of the tracer is equal to the concentration of protein at 300 nM. The competitors for binding include the ssDNA G15D and RNA 24.

Figures 11A and 11B show specificity of binding for thrombin ligands. Class I RNA 16 (SEQ ID NO:198), Class II RNA 27 (SEQ ID NO:209), and bulk 30N RNA were chosen for binding analysis with human antithrombin III (Sigma) (Figure 11A) and human prothrombin (Sigma) (Figure 11B).

Figure 12 shows the results of nitrocellulose filter binding assays for the 30N and 60N DNA candidate mixtures and the nucleic acid pools, both 30N and 60N, after performing 11 rounds of SELEX to thrombin.

Figure 13 depicts the binding curve for the truncated thrombin DNA ligand referred to as 60-18(38) (SEQ ID NO:278) and the binding curve for the non-truncated form of the same DNA ligand, 60-18 (SEQ ID NO:279).

Figure 14 depicts the results of the thrombin DNA ligand 60-18(38) (SEQ ID NO:278) in the clot inhibition assay.

#### DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

This application is an extension and an application of the method for identifying nucleic acid ligands referred to as SELEX. The SELEX method is described in detail in U.S. patent application serial number 07/141,131, filed June 10, 1991, entitled Nucleic Acid Ligands, 07/536,428, filed June 11, 1990,

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entitled Systematic Evolution of Ligands by Exponential Enrichment, now abandoned, 07/931,473 filed August 17, 1992, now United States Patent No. 5,270,163, entitled

Nucleic Acid Ligands. These applications are collectively referred to herein as the SELEX

Applications. The full text of these applications, including but not limited to, all definitions and descriptions of the SELEX process, are specifically incorporated herein by reference.

In its most basic form, the SELEX process may be defined by the following series of steps:

1) A candidate mixture of nucleic acids of differing sequence is prepared. The candidate mixture generally includes regions of fixed sequences (i.e., each of the members of the candidate mixture contains the same sequences in the same location) and regions of randomized sequences. The fixed sequence regions are selected either: a) to assist in the amplification steps described below; b) to mimic a sequence known to bind to the target; or c) to enhance the concentration of a given structural arrangement of the nucleic acids in the candidate mixture. The randomized sequences can be totally randomized (i.e., the probability of finding a base at any position being one in four) or only partially randomized (i.e., the probability of finding a base at any location can be selected at any level between 0 and 100 percent).

2) The candidate mixture is contacted with the selected target under conditions favorable for binding between the target and members of the candidate mixture. Under these circumstances, the interaction between the target and the nucleic acids of the candidate mixture can be considered as forming nucleic acid-target pairs between the target and the nucleic acids having the strongest affinity for the target.

3) The nucleic acids with the highest affinity for the target are partitioned from those

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nucleic acids with lesser affinity to the target. Because only an extremely small number of sequences (and possibly only one molecule of nucleic acid) corresponding to the highest affinity nucleic acids exist in the candidate mixture, it is generally desirable to set the partitioning criteria so that a significant amount of the nucleic acids in the candidate mixture (approximately 5-50%) are retained during partitioning.

4) Those nucleic acids selected during partitioning as having the relatively higher affinity to the target are then amplified to create a new candidate mixture that is enriched in nucleic acids having a relatively higher affinity for the target. 5) By repeating the partitioning and amplifying steps above, the newly formed candidate mixture contains fewer and fewer unique sequences, and the average degree of affinity of the nucleic acids to the target will generally increase. Taken to its extreme, the SELEX process will yield a candidate mixture containing one or a small number of unique nucleic acids representing those nucleic acids from the original candidate mixture having the highest affinity to the target molecule.

The SELEX Patent Applications describe and elaborate on this process in great detail. Included are targets that can be used in the process; methods for the preparation of the initial candidate mixture; methods for partitioning nucleic acids within a candidate mixture; and methods for amplifying partitioned nucleic acids to generate enriched candidate mixtures. The SELEX Patent Applications also describe ligand solutions obtained to a number of target species, including both protein targets wherein the protein is and is not a nucleic acid binding protein.

SELEX provides high affinity ligands of a

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target molecule. This represents a singular achievement that is unprecedented in the field of nucleic acids research.

The present invention applies the SELEX procedure to the specific targets, bFGF and thrombin. In the Example section below, the experimental parameters used to isolate and identify the nucleic acid ligand solutions to bFGF and thrombin are described.

In order to produce nucleic acids desirable for use as a pharmaceutical, it is preferred that the nucleic acid ligand 1) binds to the target in a manner capable of achieving the desired effect on the target; 2) be as small as possible to obtain the desired effect; 3) be as stable as possible; and 4) be a specific ligand to the chosen target. In most, if not all situations, it is preferred that the nucleic acid ligand have the highest possible affinity to the target.

In co-pending and commonly assigned U.S.

Patent Application Serial No. 07/954,624, filed October

21, 1992, methods are described for obtaining improved nucleic acid ligands after SELEX has been performed.

This application, entitled Methods of Producing Nucleic Acid Ligands is specifically incorporated herein by reference. Included in this application are methods relating to assays of ligand effects on target

molecules; affinity assays of the ligands; information boundaries determination; quantitative and qualitative assessment of individual nucleotide contributions to affinity via secondary SELEX; nucleotide substitution, and chemical modification experiments; and structural determination. The present invention includes improvements to the nucleic acid ligand solutions derived according to these procedures.

This invention includes the specific nucleic acid ligands shown in Tables II-IV, Table VIII, Tables XIII-XIII, Tables XV-XVIII and Tables XXI-XXII. These

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tables include unmodified RNA ligands to bFGF (Tables II-IV (SEQ ID NOS: 8-69)), modified RNA ligands to bFGF (Table VIII (SEQ ID NOS: 101-185)), DNA ligands to bFGF (Tables XXI-XXII (SEQ ID NOS: 330-445)), unmodified RNA ligands to thrombin (Tables XIII-XIII (SEQ ID NOS: 192-214)) and DNA ligands to thrombin (Tables XV-XVIII (SEQ ID NOS: 216-319)) identified by the SELEX method as described herein. The scope of the ligands covered by this invention extends to all ligands to bFGF and thrombin identified according to the SELEX procedure.

More specifically, this invention includes nucleic acid sequences that are substantially homologous to and that have substantially the same ability to bind bFGF and thrombin as the specific nucleic acid ligands shown in Tables II-IV, VIII, XIII-XIII, XV-XVIII and XXI-XXII. By substantially homologous, it is meant, a degree of primary sequence homology in excess of 70%, most preferably in excess of 80%. Substantially the same ability to bind bFGF or thrombin means that the affinity is within two orders of magnitude of the affinity of the ligands described herein. It is well within the skill of those of ordinary skill in the art to determine whether a given sequence -- substantially homologous to those specifically described herein -- has substantially the same ability to bind bFGF or thrombin.

A review of the proposed structural formations shown in Figure 4 for the Family 1 and 2 unmodified ligands to bFGF and Figure 7 for the Class 1 and 2 unmodified ligands to thrombin shows that sequences that have little or no primary sequence homology may still have substantially the same ability to bind bFGF or thrombin, respectively. It can be assumed that the disparate sequences in Figure 4 have similar structures that give rise to the ability to bind to bFGF, and that each of the Family 1 and Family 2 sequence ligands are able to assume structures that

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appear very similar to the binding site of bFGF even though they may not bind the same site. Likewise, it can be assumed that the disparate sequences depicted in Figure 7 have a common structure that gives rise to the ability to bind to thrombin, and that each of the Class 1 and Class 2 sequence ligands are able to assume structures that appear very similar to the binding site of thrombin even though they may not bind the same site. For these reasons, the present invention also includes RNA ligands that have substantially the same structure as the ligands presented herein and that have substantially the same ability to bind bFGF and thrombin as the RNA ligands shown in Tables II and III and Table XII, respectively. "Substantially the same structure" includes all RNA ligands having the common structural elements of the sequences given in Tables II, III and XII.

As stated above, this invention also includes the specific 2'-NH<sub>2</sub>-modified nucleic acid ligands to bFGF shown in Table VIII. These ligands were identified by the SELEX method utilizing a candidate mixture of RNAs wherein all pyrimidines were 2'-deoxy-2'-NH<sub>2</sub>. All purines utilized in these experiments were unmodified, or 2'-OH. More specifically, this invention includes nucleic acid sequences that are substantially homologous to and that have substantially the same ability to bind bFGF as the specific nucleic acid ligands shown in Table VIII.

This invention also covers the specific DNA nucleic acid ligands to bFGF (Tables XVI and XVII) and thrombin (Tables XV and XVI). Also included are DNA sequences that are substantially homologous to and that have substantially the same ability to bind thrombin and bFGF as the specific sequences given in Tables XV, XVI, XXI and XXII. Also included are DNA ligands that have substantially the same structure as the ligands presented in Tables XV, XVI, XXI and XXII and that have

substantially the same ability to bind thrombin and bFGF, respectively.

This invention also includes the ligands described above, wherein certain chemical modifications have been made in order to increase the *in vivo* stability of the ligand, enhance or mediate the delivery of the ligand, or reduce the clearance rate from the body. Examples of such modifications include chemical substitutions at the ribose and/or phosphate and/or base positions of a given RNA sequence. See, e.g., Cook et al. PCT Application WO 92/03568; U.S. Patent No. 5,118,672 of Schinazi et al.; Hobbs et al. (1973) Biochem. 12:5138; Guschlauer et al. (1977) Nucleic Acids Res. 4:1933; Shibabara et al. (1987) Nucleic Acids Res. 15:4405; Pieken et al. (1991) Science 253:314, each of which is specifically incorporated herein by reference. Such modifications may be made post-SELEX (modification of previously identified unmodified ligands) or by incorporation into the SELEX process as described below.

Two SELEX experiments were conducted to select unmodified RNA ligands to bFGF (Examples 1 and 2). These experiments yielded two sequence families of high-affinity nucleic acid ligands to bFGF Family 1 and Family 2 (Tables II and III), as well as single sequences ("other sequences") (Table IV) and repeat sequences (Table V). A review of the two sequence families (Tables II and III) shows that sequences that have little or no primary sequence homology may still have substantially the same ability to bind bFGF. It appears that the disparate sequences may have a common structure that gives rise to the ability to bind to bFGF, and that each of the sequence Family 1 and 2 ligands are able to assume structures that appear very similar to the binding site of bFGF even though they may not bind the same site. High-affinity nucleic acid ligands selected in the presence of heparin (Experiment

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B) exhibited the consensus sequence of Family 2. These ligands bind a bFGF protein in which a conformation change has been induced by heparin.

The high-affinity nucleic acid ligands to bFGF of the present invention may also have various properties, including the ability to inhibit the biological activity of bFGF. Representative ligands from Family 1 and 2 (Tables II and III) were found to inhibit binding of bFGF to both low-and high-affinity cell-surface receptors (Example 5). These nucleic acid ligands may be useful as specific and potent neutralizers of bFGF activity *in vivo*.

Two SELEX experiments, to select ligands to bFGF, were conducted with RNA candidate mixtures wherein all pyrimidine moieties were 2'-deoxy-2'-NH<sub>2</sub>-pyrimidines (Example 4, experiments A and B). These experiments yielded the sequences shown in Table VIII. Sequence families 1A, 1B, 1C, 2 and 3 were identified, as well as, four families containing two sequences each ("two-member families"), single sequences ("other sequences"), and sequences binding nitrocellulose ("nitrocellulose-binding family"). The nitrocellulose-binding ligands had an increased affinity to nitrocellulose as well as an increased affinity to bFGF. The high affinity of identified 2'-NH<sub>2</sub> ligands for bFGF is shown in Table IX and Figure 5. 2'-NH<sub>2</sub>-modified RNA ligands able to inhibit the *in vitro* activity of bFGF were identified (Figure 6). These ligands were shown to inhibit the biological activity of bFGF *in vivo* (Example 6).

The effect of the modified 2'-NH<sub>2</sub> RNA ligands on endothelial cell motility was examined in Example 7. Ligand 21A-ts (SEQ ID NO:44), a chemically synthesized analogue of ligand 21A-t (SEQ ID NO:186), was found to inhibit bovine aortic endothelial (BAE) cell migration in a dose dependent manner at concentrations greater than 50 nM. The total amount of motility that could be

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inhibited by 21A-ts at high concentrations was comparable in all experiments to the effect of 100 µg/ml neutralizing bFGF antibody.

Example 8 describes the evolution of high affinity DNA ligands to bFGF using SELEX (see Table XXI). Candidate mixtures with 30 and 40 variable nucleotide regions were employed in three experiments starting with three separate sets of synthetic DNA oligonucleotide templates and primers (see Table XIX). A significant improvement in affinity of DNA ligands to bFGF was observed in each of the three experiments after ten rounds of selection (see Table XX in which the results for Experiment 3 are depicted). Five distinct families were identified based on 40% or better overlap in sequence homology (Table XXI). A number of sequences with no homology to members of the five families were also present and are listed in Table XXI as orphans.

A majority of the ligands isolated from Experiments 1 and 3 were screened for their ability to bind bFGF and high-affinity ligands for bFGF were found in five sequence families (see Example 8 and Table XXI (\*)). The Kds of the isolates tested for affinity to bFGF are listed in Table XXII. Removal of nucleotides non-essential for binding was performed on five of the ligands with the highest affinity for bFGF, Kds less than 1 nM (Table XXII, Truncations).

The five truncated molecules were tested for their ability to inhibit binding of bFGF to its low- and high-affinity cell-surface receptors. All five ligands show inhibition in the nanomolar range. Truncated Ligand M225t3 (SEQ ID NO:364) was also tested for its specificity. It was found that the affinity of M225t3 for vasoconstrictor endothelial growth factor and human chorionic gonadotropin, two heparin-binding proteins, was relatively weak (Kd > 0.2 µM).

To determine whether enhanced circulation

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time could be obtained by conjugating the bFGF ligand to a high molecular weight species, a M225t3 DNA ligand was synthesized and coupled with an N-hydroxysuccinimidyl active ester of PEG 3400 (Example 9). The PEG modified M225t3 was shown to bind bFGF with a similar affinity as the non-modified ligand.

The nucleic acid ligands and nucleic acid ligand solutions to bFGF described herein are useful as pharmaceuticals, and as part of gene therapy treatments. Example 6 shows the ability of 2'-NH<sub>2</sub>-modified RNA ligands to inhibit the *in vivo* biological activity of bFGF. Further, the nucleic acid ligands to bFGF described herein may be used beneficially for diagnostic purposes.

The SELEX process for identifying ligands to

a target was performed using human thrombin as the target, and a candidate mixture containing 76 nucleotide RNAs with a 30 nucleotide region of unmodified randomized sequences (Example 10).

Following twelve rounds of SELEX, a number of the selected ligands were sequenced, to reveal the existence of two groups of sequences that had common elements of primary sequence (Example 11).

A dramatic shift in binding of the RNA population was observed after 12 rounds of SELEX, when compared to the bulk 30N RNA. Sequencing of bulk RNA after 12 rounds also showed a non-random sequence profile. The RNA was reverse transcribed, amplified, cloned and the sequences of 28 individual molecules were determined (Table XII). Each sequence is divided into 3 blocks from left to right: 1) the 5' fixed region, 2) the 30N variable region, and 3) the 3' fixed region. Based on primary sequence homology, 22 of the RNAs were grouped as Class I and 6 RNAs were grouped as Class II. Of the 22 sequences in Class I, 16 (8 of which were identical) contained an identical sequence motif GGAUCGAG (N) AGUAGGC (SEQ ID NO:190), whereas the

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remaining 6 contained 1 or 2 nucleotide changes in the defined region or some variation in N=2 to N=5. This conserved motif varied in its position within the 30N region. In Class II, 3 of the 6 RNAs were identical and all of them contained the conserved motif

GCGCTTGGAGCCUGCU (SEQ ID NO:191), beginning at the 3rd nucleotide from the end of the 5' fixed region.

Three sequence variant RNA ligands from Class I (6 (SEQ ID NO:192), 16 (SEQ ID NO:198), and 18 (SEQ ID NO:199)) and one (27 (SEQ ID NO:209)) from Class II, identified by the order they were sequenced, were used for individual binding analysis. Class I RNAs were exemplified by clone 16 with a Kd of approximately 30 nM and the Kd for the Class II RNA clone 27 was

approximately 60 nM.

In order to identify the minimal sequence requirements for specific high affinity binding of the 76 nucleotide RNA which includes the variable 30N region flanked by 5' and 3' fixed sequence, 5' and 3' boundary experiments were performed (Example 12). For 5' boundary experiments the RNAs were 3' end labeled and hydrolyzed to give a pool of RNAs with varying 5' ends. For the 3' boundary experiments, the RNAs were 5' end-labeled and hydrolyzed to give a pool of RNAs with varying 3' ends. Minimal RNA sequence requirements were determined following RNA protein binding to nitrocellulose filters and identification of labeled RNA by gel electrophoresis (Example 12). 3' boundary experiments gave the boundaries for each of the 4 sequences shown in Table XIII. These boundaries were consistent at all protein concentrations. 5' boundary experiments gave the boundaries shown in Table XIII plus or minus 1 nucleotide, except for RNA 16 which gave a greater boundary with lower protein concentrations. Based on these boundary experiments, possible secondary structures of the thrombin ligands are shown in Figure

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7. RNAs corresponding to the smallest and largest hairpin of Class I clone 16 (SEQ ID NO:212) (24 and 39 nucleotides) and the hairpin of Class II clone 27 (SEQ ID NO:214) (33 nucleotides) were synthesized or transcribed for binding analysis (see Figure 7 and Example 13). Results show that the RNA 27 hairpin binds with affinity ( $K_d$  of about 60 nM) equal to that of the entire 72 nucleotide transcript with fixed and variable region (compare RNA 27 in Figure 8A with RNA 33R in Figure 8C). The  $K_d$ s for Class I clone 16 RNA hairpins on the other hand increased an order of magnitude from 30 nM to 200 nM.
- Modifications in the 2'-NH<sub>2</sub>-ribose of pyrimidine residues of RNA molecules has been shown to increase stability of RNA (resistant to degradation by RNase) in serum by at least 1000 fold. 2'-NH<sub>2</sub> modified RNAs were prepared in Example 14. Binding experiments (Example 14) with the 2'-NH<sub>2</sub>-GTP/UTP modified RNAs of Classes I and Class II showed a significant drop in binding when compared to the unmodified RNA (Figure 9). Binding by the bulk 3'N RNA, however, showed a slight increase in affinity when it was modified.
- A ssDNA molecule with a 15 nucleotide consensus 5'-GGTTGAGTCATGG-3' (G15D) (SEQ ID NO:189) has been shown to bind human thrombin and inhibit fibrin-clot formation in vitro (Bock et al. (1992) Nature 355:564-565). The results of competition experiments for binding thrombin between G15D and the RNA hairpin ligands of this invention are shown in Figure 10 (see Example 15). In the first of these experiments (Experiment A) a <sup>32</sup>P-labeled G15D was used as the tracer with increasing concentrations of unlabeled RNA or unlabeled G15D. As expected, when the G15D was used to compete for its own binding, binding of labeled DNA was reduced to 50% at equimolar concentrations (1  $\mu$ M) of labeled and unlabeled

competitor DNA. Both the Class I clone 16 synthetic RNAs 24 and 39, and the Class II clone 27 synthetic RNA 33 were able to compete for binding of G15D at this concentration. In the second experiment (Experiment B) the higher affinity Class II hairpin RNA 33 ( $K_d$  = 60 nM) was <sup>32</sup>P-labelled and used as the tracer with increasing concentrations of unlabeled RNA or unlabelled G15D DNA ( $K_d$  = 200 nM). In these experiments, the G15D was able to compete effectively with RNA 33 at higher concentrations than the RNA 33 competes itself (shift of binding to the right), which is what is expected when competing with a ligand with 3-4 fold higher affinity. The Class II hairpin RNA 33 ( $K_d$  = 60 nM) was competed only weakly by the class I hairpin RNA 24 ( $K_d$  = 200 nM), suggesting that while there may be some overlap, the RNAs of these two classes may bind with high affinity to different yet adjacent or overlapping sites. Because both of these RNAs can compete for G15D binding, this DNA isomer probably binds in the region of overlap between the Class I and Class II hairpins.

The ability of thrombin to cleave the peptidyl chromogenic substrate S2238 (H-D-Phe-Pip-Arg-pNitroaniline) (H-D-Phe-Pip-Arg-pNA) (Rabi Pharma) was measured in the presence and absence of the RNA ligands of this invention (Example 16). The hydrolysis by thrombin of the chromogenic substrate S-2238 (H-D-Phe-Pip-Arg-pNitroaniline) at the indicated thrombin and RNA concentration was measured photometrically at 405 nm (Table XIV). There was no inhibitory effect of RNA on this cleavage reaction at 10<sup>-4</sup> M thrombin and 10<sup>-4</sup> M RNA, 10<sup>-4</sup> M thrombin and 10<sup>-4</sup> M RNA or at 10<sup>-4</sup> M thrombin and 10<sup>-3</sup> M RNA. These results suggest that the RNA ligands do not bind in the catalytic site of the enzyme.

The ability of thrombin to catalyze clot formation by cleavage of fibrinogen to fibrin was

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measured in the presence and absence of RNA (Example 17). The conversion of fibrinogen to fibrin and resulting clot formation was measured by the tilt test in the presence and absence of the RNA ligand inhibitors described. When RNA was present at a concentration equal to the  $K_d$  (30 nM for Class I RNAs and 60 nM for Class II RNAs), which was in 5 to 10-fold excess of thrombin, clotting time was increased by 1.5-fold (Table XIV).

Representative ligands from Class I and Class II showed that these ligands had low affinity for ATIII at concentrations as high as 1  $\mu$ M (Example 18, Figure 11A). These ligands showed reduced affinity when compared with the bulk 30n3 RNA suggesting that there has been selection against non-specific binding. This is of particular importance because ATIII is an abundant plasma protein with high affinity for heparin, a polyanionic macromolecule. These results show that the evolution of a discreet structure present in the Class I and Class II RNAs is specific for thrombin binding and, despite its polyanionic composition, does not bind to a high affinity heparin binding protein.

It is also important to note that these thrombin specific RNA ligands have no affinity for prothrombin (Example 18, Figure 11B), the inactive biochemical precursor to active thrombin, which circulates at high levels in the plasma (~ 1  $\mu$ M). Example 19 (Table XV) below describes the evolution of high affinity DNA ligands to thrombin utilizing SELEX. Candidate mixtures with 30 and 60 variable nucleotide regions were employed in separate experiments. The binding constants of several of the ligands to thrombin were obtained, and one of the ligands 60-18(38) (SBQ ID No:279) was shown to inhibit coagulation by thrombin (Table XVI).

The nucleic acid ligands and nucleic acid ligand solutions to thrombin described herein are

useful as pharmaceuticals and as part of gene therapy treatments. The ligands can also be useful for diagnostic purposes.

The concepts of vascular injury and thrombosis are important in the understanding of the pathogenesis of various vascular diseases, including the initiation and progression of atherosclerosis, the acute coronary syndromes, vein graft disease, and restenosis following coronary angioplasty.

The high-affinity thrombin binding RNA ligands of this invention may be expected to have various properties. These characteristics can be thought about within the context of the hirudin peptide inhibitors and the current understanding of thrombin structure and binding. Within this context and not being limited by theory, it is most likely that the RNA ligands are binding the highly basic anionic exosite. It is also likely that the RNA is not binding the catalytic site which has high specificity for the cationic arginine residue. One would expect the RNA ligands to behave in the same manner as the C-terminal hirudin peptides. As such, they would not strongly inhibit small peptidyl substrates, but would inhibit fibrinogen-clotting, protein C activation, platelet activation, and endothelial cell activation. Given that within the anionic exosite the fibrinogen-clotting and Tm-binding activities are separable, it is possible that different high-affinity RNA ligands may inhibit these activities differentially. Moreover, one may select for one activity over another in order to generate a more potent anticoagulant than procoagulant.

#### EXAMPLE 1. EXPERIMENTAL PROCEDURES.

**Materials.** dGDP was obtained from Bachem California (molecular weight 19,000 Da, 154 amino acids). Tissue culture grade heparin (average molecular weight 16,000 Da) was purchased from Sigma.

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Low molecular weight heparin (5,000 Da) was from Calbiochem. All other chemicals were at least reagent grade and were purchased from commercial sources.

**SELEX.** Evolution of High Affinity Ligands to bFGF. Essential features of the SELEX protocol have been described in detail in the SELEX Applications and in previous papers (Tuerk & Gold (1990) *Science* 242:505; Tuerk et al. (1992a) *Proc. Natl. Acad. Sci. USA* 89:6988; Tuerk et al. (1992b) in Polymerase Chain Reaction (Ferre, F., Mullis, K., Gibbs, R. & Ross, A., eds.) Birthhäuser, NY). The SELEX protocol may be performed in generally the same manner for unmodified RNA selection as for selection with 2'-NH<sub>2</sub> pyrimidines as described in Example 4 below. Briefly, DNA templates for in vitro transcription (that contain a region of thirty random positions flanked by constant sequence regions) and the corresponding PCR primers were synthesized chemically (Operon). The random region was generated by utilizing an equimolar mixture of the four nucleotides during oligonucleotide synthesis. The two constant regions were designed to contain PCR primer annealing sites, a primer annealing site for cDNA synthesis, T7 RNA polymerase promoter region, and restriction enzyme sites that allow cloning into vectors (see Table 1).

An initial pool of RNA molecules was prepared by in vitro transcription of about 200 picomoles (pmol) ( $10^{14}$  molecules) of the double stranded DNA template (10<sup>14</sup> molecules) utilizing T7 RNA polymerase (New England Biolabs).

Transcription mixtures consisted of 100-300 nM template, 5 units/ $\mu$ l T7 RNA polymerase, 40 mM Tris-Cl buffer (pH 8.0) containing 12 mM MgCl<sub>2</sub>, 5 mM DTT, 1 mM spermidine, 0.002% Triton X-100, and 4% PEG. Transcription mixtures were incubated at 37 °C for 2-3 hours. These conditions typically resulted in transcriptional amplification of 10- to 100-fold.

Selections for high affinity RNA ligands to

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bFGF were done by incubating bFGF (100-100 pmol) with RNA (90-300 pmol) for 10 minutes at 37 °C in 50  $\mu$ l of phosphate buffered saline (PBS) (10.1 mM Na<sub>2</sub>HPO<sub>4</sub>, 1.8 mM KH<sub>2</sub>PO<sub>4</sub>, 137 mM NaCl, 2.7 mM KCl, pH 7.4), then separating the protein-RNA complexes from the unbound species by nitrocellulose filter partitioning (Tuerk & Gold (1990) *Science* 242:505). The selected RNA (which typically amounts to 0.3-8% of the total input RNA) was then extracted from the filters and reverse transcribed into cDNA by avian myeloblastosis virus reverse transcriptase (AMV RT, Life Sciences). Reverse transcriptions were done at 48 °C (30 minutes) in 50 mM Tris buffer (pH 8.3), 60 mM NaCl, 6 mM Mg(OAc)<sub>2</sub>, 10 mM DTT, and 1 unit/ $\mu$ l AMV RT. Amplification of the cDNA by PCR under standard conditions yielded sufficient amounts of double-stranded DNA for the next round of in vitro transcription.

**Nitrocellulose Filter Binding Assay.**

Oligonucleotides bound to proteins can be effectively separated from the unbound species by filtration through nitrocellulose membrane filters (Varus & Berg (1970) *Anal. Biochem.* 35:450; Lowary & Uhlenbeck (1987) *Nucleic Acids Res.* 15:10483; Tuerk & Gold (1990) *Science* 242:503). Nitrocellulose filters (Millipore, 0.45  $\mu$ m pore size, type HM) were secured on a filter manifold and washed with 4-10 ml of buffer. Following incubations of <sup>32</sup>P-labeled RNA with serial dilutions of the protein (5-10 min) at 37 °C in buffer (PBS) containing 0.01% human serum albumin (HSA), the solutions were applied to the filters under gentle vacuum in 45  $\mu$ l aliquots and washed with 5 ml of PBS. The filters were then dried under an infrared lamp and counted in a scintillation counter.

**Cloning and Sequencing.** Individual members of the enriched pools were cloned into pUC18 vector and sequenced as described (Schneider et al. (1992) *J. Mol. Biol.* 228:862-869; Tuerk & Gold (1990) *supra*).

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**EXAMPLE 2. SELEX EXPERIMENTS TARGETING bFGF.**

Following the procedures described in Example 1 above, two SELEX experiments (Experiments A and B) targeting bFGF were initiated with separate pools of randomized unmodified RNA, each pool consisting of approximately  $10^{14}$  molecules. The constant sequence regions that flank the randomized region, along with the corresponding primers, were different in each experiment. The two template/primer combinations used are shown in Table I.

Selections were conducted in PBS at 37 °C. The selection conducted in Experiment B was done in the presence of heparin (Sigma, molecular weight 5,000-32,000 Da, average molecular weight 16,000 Da) in the selection buffer at the molar ratio of 1/100 (heparin/bFGF). Heparin competes for binding of randomized RNA to bFGF. The amount of heparin used significantly reduced, but did not eliminate RNA binding to bFGF (data not shown). The rationale for using heparin was two-fold. First, heparin is known to induce a small conformational change in the protein and also stabilizes bFGF against thermal denaturation. Second, the apparent competitive nature of binding of heparin with randomized RNA to bFGF was expected to either increase the stringency of selection for the heparin binding site or direct the binding of RNA ligands to alternative site(s).

Significant improvement in affinity of RNA ligands to bFGF was observed in Experiment A after ten rounds, and in Experiment B after thirteen rounds. Sequencing of these enriched pools of RNA ligands revealed a definite departure from randomness which indicated that the number of different molecules remaining in the pool was substantially reduced. Individual members of the enriched pools were then cloned into pUC18 vector and sequenced as described in Example 1.

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49 clones were sequenced from Experiment A, and 37 clones from Experiment B. From the total of 86 sequences, 71 were unique. Two distinct families could be identified based on overlapping regions of sequence homology (Tables II and III, XVII and XVIII). A number of sequences with no obvious homology to members of either of the two families were also present, as expected (Irvine et al. (1991) J. Mol. Biol. 222:739), and are shown in Table IV.

The consensus sequence from Family 1 ligands (Table II) is defined by a contiguous stretch of 9 bases, CUCACCGG (SEQ ID NO:7). This suggests a minimal structure consisting of a 4-5 nucleotide loop that includes the strongly conserved ACC sequence and a bulged stem (Figure 4 and Table VI). The consensus sequence for Family 2 ligands (Table III) is more extended and contains less conserved regions, RGGHACGYNNGDCHAGNNCACTY (SEQ ID NO:23). Here, most of the strongly conserved positions are accommodated in a larger (19-21 nucleotide) loop (Figure 4 and Table VII). Additional structure within the loop is possible.

The existence of two distinct sequence families in the enriched pools of RNA suggest that there are two convergent solutions for high-affinity binding to bFGF. SELEX Experiment A contributed members to both sequence families (Table II). All of the sequences from the SELEX Experiment B (selected in the presence of heparin), on the other hand, belong either to Family 2 (Table III) or to the "other sequences" family (Table IV), but none were found in Family 1. This is surprising in view of the fact that bFGF was present in a molar excess of 100-fold over heparin during selections. The effective molar excess of bFGF over heparin, however, was probably much smaller. Average molecular weight of heparin used in selections was 16,000 Da. Since each sugar unit weighs

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320 Da and at least eight sugar units are required for high-affinity binding to bFGF; six molecules of bFGF, on average, can bind to a molecule of heparin. This reduces the molar ratio of heparin to bFGF to 1:16. In practice, this amount of heparin is sufficient to reduce the observed affinity of the unselected RNA pool for bFGF by a factor of five (data not shown). The observed exclusion of an entire ligand family by the presence of a relatively small amount of heparin in the selection buffer may be a consequence of a conformational change in the protein induced by heparin. Because of the relative amounts of heparin and bFGF that were used in selections, this model may require that the heparin-induced conformation persist after the protein-heparin complex has dissociated, and that the lifetime of this conformer is long enough to permit equilibration with the RNA ligands.

Family 2 sequences are comprised of clones derived from both SELEX experiments. This suggests that the flanking constant regions typically play a relatively minor role in determining the affinity of these ligands and supports the premise that the consensus sequence in this family is the principal determinant of high-affinity binding to bFGF.

#### EXAMPLE 3. DETERMINATION OF BINDING AFFINITIES FOR bFGF.

##### Equilibrium Dissociation Constants.

In the simplest case, equilibrium binding of RNA to bFGF can be described by equation 1:



The fraction of bound RNA ( $q$ ) is related to the concentration of free protein,  $[P]$  (equation 2):

$$q = f[P] / [P] + K_d \quad (2)$$

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where  $K_d$  is the equilibrium dissociation constant and  $f$  reflects the efficiency of retention of the protein-RNA complexes on nitrocellulose filters. Mean value of  $f$  for bFGF was 0.82.

In order to eliminate higher order structures, all RNA solutions were heated to 90 °C in PBS for 2-3 minutes and cooled on ice prior to incubation with protein. Only single bands for all RNA clones were detected on non-denaturing polyacrylamide gels following this treatment.

Relative binding affinity of individual ligands to bFGF cannot be predicted from sequence information. Unique sequence clones were therefore screened for their ability to bind to bFGF by measuring the fraction of radiolabeled RNA bound to nitrocellulose filters following incubation with 4 and 40 nM protein. This screening method was sufficiently accurate to allow several clones to be identified that had dissociation constants in the nanomolar range. Binding of these select clones was then analyzed in more detail.

High-affinity RNA ligands for bFGF were found in both sequence families (Tables VI and VII). The affinity of clones that did not belong to either family was generally lower (data not shown).

The original, unselected RNA pools bound to bFGF with 300 nM (set A) and 550 nM (set B) affinities (Figure 1). SELEX therefore allowed the isolation of ligands with at least 2 orders of magnitude better affinity for bFGF.

In order to address the question of specificity, a representative set of high-affinity ligands for bFGF (5A (SEQ ID NO:9) and 7A (SEQ ID NO:10) from Family 1; 12A (SEQ ID NO:25) and 26A (SEQ ID NO:26) from Family 2) were tested for binding to four other heparin-binding proteins. It was found that the affinity of these ligands for acidic FGF, thrombin,

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antithrombin III, and vascular endothelial growth factor was relatively weak ( $K_d > 0.3 \mu\text{M}$ ) (data not shown).

**EXAMPLE 4. MODIFIED 2'-NH<sub>2</sub>-PYRIMIDINE RNA LIGANDS TO bFGF.**

In order to generate ligands with improved stability *in vivo*, two SELEX experiments (A and B) targeting bFGF were initiated with separate pools of randomized RNA containing amino ( $\text{NH}_2$ ) functionalities at the 2' position of each pyrimidine. Starting ligand pools for the two experiments contained approximately  $10^{14}$  molecules (500 pmols) of modified RNA randomized at 30 (SELEX experiment A) and 50 (SELEX experiment B) contiguous positions. The starting RNAs and the corresponding PCR primers are defined in Table XI. Following twelve rounds of SELEX, the affinity of the modified RNA pools was improved by 1-2 orders of magnitude. Sequences corresponding to the evolved regions of modified RNA are shown in Table VIII. It is interesting to note that individual nucleotides occur at substantially different frequencies with guanine being conspicuously overrepresented (43%), adenine and uridine occurring at about equal frequencies (22% and 21%) and cytosine being underrepresented (14%).

Groups of ligand sequences with similar primary structure (families) have been aligned in Table VIII and their consensus sequences are shown below each set. Pairs of similar/related sequences, sequences that could not be included in any of the families ("other sequences") and sequences that correspond to ligands that bind additionally to nitrocellulose filters with high affinity have been shown in separate groups. The letter N in a sequence indicates an ambiguous position on a sequencing gel. An italicized letter N in a consensus sequence indicates a position that is not conserved (i.e., any nucleotide may be

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found at that position).

All unique ligands were screened for their binding affinities for bFGF by measuring the fraction of RNA bound to bFGF at two protein concentrations (5.0 and 0.5 nM bFGF). This affinity screening allowed identification of those ligands with highest affinity for bFGF. Binding of a group of these ligands was analyzed over a range of bFGF concentrations (Figure 5) and their dissociation constants ( $K_d$ 's) were determined as described (Jallinek et al. (1993) Proc. Natl. Acad. Sci. USA 90:11227-11231) (Table IX). RNA concentrations were determined from their absorbance reading at 260 nm (and were typically  $<100 \mu\text{M}$ ). Binding reactions were done at 37 °C in phosphate buffered saline containing 0.01% human serum albumin and 1 mM DTT.

The minimal sequence information required for high-affinity binding to bFGF was examined for several of the 2'-NH<sub>2</sub> modified ligands by deletion analyses as described (Turk et al. (1990) J. Mol. Biol. 213:749-761). Truncated ligands 21A-t (GGUURGUGANGAGCAGCGGAGUC (SEQ ID NO:186)), the letter "t" is used to designate truncated sequences derived from the corresponding parent sequences; underlined G's are those guanine nucleotides added to improve the efficiency of transcription; lowercase letters are from the constant sequence region), 58A-t (GGACGCCGCGCCGCGGCG) (SEQ ID NO:187) and 34B-t (GggagacgaaugcgaaGggggcgc (GGCGCGGC) (SEQ ID NO:188) were synthesized enzymatically using T7 RNA polymerase from synthetic DNA templates and their binding affinity for bFGF was examined. Ligand 21A-t binds to bFGF in a biphasic manner with a dissociation constant of the higher affinity component ( $K_{d1}$ ) of 0.1 nM, mole fraction of the higher affinity component ( $x_1$ ) of 0.5 and a dissociation constant of the lower affinity component ( $K_{d2}$ ) of 270 nM (for interpretation

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of biphasic binding see Jellinek et al. (1993) Proc. Natl. Acad. Sci. USA 90:11227-11231). Binding of ligand 58A-t to bFGF is also biphasic ( $Kd1 = 1.8 \text{ nM}$ ,  $\chi_1 = 0.5$ ,  $Kd2 = 180 \text{ nM}$ ). Binding of ligand 34B-t is monophasic ( $Kd1 = 3 \text{ nM}$ ).

The ability to inhibit the binding of  $^{125}\text{I}$ -bFGF to high and low-affinity cell-surface receptors was examined (Figure 6). Experiments were conducted as described in Moscatelli (1987) J. Cell. Physiol. 131:123 using confluent cultures of baby hamster kidney cells. Specific activity of bFGF was 915 cpm/fmol.

Each data point represents the average of two experiments.

Several high-affinity ligands were found to inhibit binding of bFGF to its cell-surface receptors, with truncated versions of ligand 21A being the most effective inhibitors (Figure 6B). Random RNA was ineffective in this concentration range (up to 1  $\mu\text{M}$ ).

#### EXAMPLE 5. RNA LIGAND INHIBITION OF bFGF RECEPTOR BINDING.

The same four high-affinity RNA ligands (5A (SEQ ID NO:9) and 7A (SEQ ID NO:10) from Family 1, 12A (SEQ ID NO:25) and 26A (SEQ ID NO:26) from Family 2) described in Example 3 were also tested for their ability to inhibit binding of bFGF to the low- and the high-affinity cell-surface receptors. Additionally, modified RNA ligands 21A (SEQ ID NO:104), 38B (SEQ ID NO:114) and random RNAs were tested.

**Receptor Binding Studies.** bFGF was labeled with  $^{125}\text{I}$  by the Iodo-Gen (Pierce) procedure as described by Moscatelli (1987) J. Cell. Physiol. 131:123. Confluent baby hamster kidney (BHK) cells were washed extensively with PBS and then incubated for 2 hours at 4°C with qMEM medium containing 10 ng/ml  $^{125}\text{I}$ -bFGF in PBS, 0.1% HSA, 1 unit/ml RNasein, and serial dilutions of high-affinity RNA. In a separate

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experiment it was established that the RNA is not significantly degraded under these conditions. The amount of  $^{125}\text{I}$ -bFGF bound to the low- and the high-affinity receptor sites was determined as described by Moscatelli (1987) *supra*.

All four ligands competed for the low-affinity receptor sites while the unselected (random) RNAs did not (Figure 2A). The concentration of RNA required to effect half-displacement of bFGF from the low-affinity receptor was 5-20 nM for ligands 5A, 7A and 26A, and >100 nM for ligand 12A. Half-displacement from the high-affinity sites is observed at the concentration of RNA near 1  $\mu\text{M}$  for ligands 5A, 7A and 26A, and > 1  $\mu\text{M}$  for ligand 12A (Figure 2B). Again, random RNAs did not compete for the high-affinity receptor. The observed difference in concentration of RNA required to displace bFGF from the low- and high-affinity receptors is expected as a reflection of the difference in affinity of the two receptor classes for bFGF (2-10 nM for the low-affinity sites and 10-100  $\mu\text{M}$  for the high-affinity sites).

Binding curves for modified RNA ligands 21A (SEQ ID NO:104), 38B (SEQ ID NO:114) and random RNAs were determined (Figure 5). RNA concentrations were determined from their absorbance reading at 260 nm and were typically less than 100 pM. Binding reactions were conducted at 37 °C in phosphate buffered saline containing 0.01% human serum albumin and 1 mM DTT. Heparin competitively displaced RNA ligands from both sequence families (Figure 3), although higher concentrations of heparin were required to displace members of Family 2 from bFGF.

The selective advantage obtained through the SELEX procedure is based on affinity to bFGF. RNA ligands can in principle bind to any site on the protein, and it is therefore important to examine the activity of the ligands in an appropriate functional

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assay. The relevant functional experiment for the selected high-affinity ligands is testing their ability

to inhibit binding of bFGF to its cell-surface receptors since this is how bFGF exerts its biological activity.

The fact that several representative high-affinity RNA ligands inhibited binding of bFGF to both receptor classes (in accord with their relative binding affinities) suggests that these ligands bind at or near the receptor binding site(s). Further support for this

notion comes from the observation that heparin competes for binding of these ligands to bFGF. High affinity ligands from Family 1 and Family 2 may bind to

different sites on bFGF. This invention includes covalently connecting components from the two ligand families into a single, more potent inhibitor of bFGF.

**EXAMPLE 6. IN VIVO INHIBITION OF bFGF ACTIVITY WITH 2'-NH<sub>2</sub>-MODIFIED RNA LIGANDS.**

The potential in vivo activity of the bFGF antagonist oligonucleotide 2'-NH<sub>2</sub> ligand 21A (SBQ ID NO:104) was evaluated in the rat corneal angiogenesis assay. The basic approach for this assay was

originally developed and reported by Gimbrone et al. (1974) JNCI 52:413-419 using rabbit corneas for implantation of tumor cells or tumor cell extracts in polyacrylamide gel. The technique was later refined by Langer and Folkman (1976) Nature 263:797 to utilize a less irritating polymer, hydroxyethylmethacrylate (Hydron). The corneal implantation method for assessing angiogenic activity associated with cell extracts or growth factors suspended in Hydron has been used in guinea pigs by Polverini et al. (1977) Nature 262:804 and more recently in rats by Koch et al. (1992) Science 258:1798.

The corneal angiogenesis assay used herein is a modification of the techniques described in the above references. The assay is conducted in rat corneas;

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however, the implantation method is different in that the corneal pocket is made using small scissors instead

of a spatula for the blunt dissection of the corneal stroma. Additionally, Hydron could not be used as the carrier substance for bFGF because the protein was denatured by the high concentration of ethanol and/or

the polymerization reaction. Other carriers were studied and it was determined that nitrocellulose filter material (Millipore) was the most suitable

medium for implantation since it readily absorbs the protein, is not denaturing to proteins, and is not proinflammatory or irritating to the corneal stroma.

The basic design of the first in vivo assay was to compare the potential angiogenic effects of (1) untreated nitrocellulose, (2) nitrocellulose soaked in oligonucleotide 2'-NH<sub>2</sub>, ligand 21A, (3) nitrocellulose soaked in bFGF, and (4) nitrocellulose soaked in a solution of ligand 21A and bFGF combined.

The disks to be implanted were punched out of a standard Millipore nitrocellulose filter using a punch made from a 16 gauge hypodermic needle. The diameter of the implanted disks was approximately 1mm. Prior to implantation the disks were soaked in a given test solution for at least one hour to ensure saturation. The four solutions in this experiment were (1) Ringer's physiologic salt solution, (2) RNA ligand 21A in 10<sup>-6</sup> PBS/0% water, (3) bFGF in Ringer's solution, and (4) 1:1 mixture of ligand 21A and bFGF.

The respective soaked disks were implanted into the corneal stroma of three rats for each treatment group. Both eyes of each rat received the same treatment so that there were six test eyes in each test group. The test solutions were handled using sterile technique. The animals were anesthetized with a general anesthetic mixture containing acepromazine, ketamine, and xylazine. The corneal surgery, which involved making an incision through the corneal

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epithelium into the underlying stroma with subsequent dissection of a pocket in the stroma, was conducted under a stereomicroscope. The surgical site was cleaned with a dilute solution of organic iodine. A single dose of ophthalmic antibiotic was administered post-surgically.

Following implantation of the disks, the animals were returned to their cages where they were maintained under standard husbandry conditions until their eyes were examined stereomicroscopically on post-surgical days seven and fourteen. The eyes were evaluated for amount of corneal cloudiness around the implant and for amount of vascular ingrowth into the normally avascular cornea. The scoring system used for quantitation of vascular ingrowth was based on degrees of vascularization around the circumference of the cornea (potential total = 360°) multiplied by the extent of vascular ingrowth toward the implant (1 = no growth; 2 = ingrowth 1/3 of distance to implant; 3 = ingrowth 2/3 of distance to implant; 4 = ingrowth to implant; 5 = ingrowth into and around implant). The mean score of the eyes in each group was then determined. The minimum score of 160 (360 x 1) is normal while the maximum possible score with extensive vascular ingrowth into the implant is 1800 (360 x 5). The results are shown in Table X.

The results from this preliminary experiment provide two important findings for this ligand. First, although the ligand did not prevent the bFGF stimulated ingrowth of vessels into the cornea (Group IV vs. Group III), it did diminish the amount of vascular ingrowth, as well as, the amount of corneal cloudiness observed microscopically at both seven and fourteen days following implantation. Second, the introduction of the oligonucleotide alone (Group II) into the cornea did not result in any adverse effects such as irritation, inflammation, or angiogenesis. These

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findings suggest that the oligonucleotide has the desired antagonistic effect for bFGF and that it is biocompatible when administered *in vivo* at relatively high local concentration (60  $\mu$ M).

#### EXAMPLE 7. ENDOTHELIAL CELL MIGRATION ASSAY.

The effect of minimal 2'-aminopyrimidine RNA ligand on endothelial cell motility was examined by measuring the migration of endothelial cells into a denuded area (Sato, Y. and Rifkin, D. B. (1989) *J. Cell Biol.* 108:309-315). Confluent monolayers of bovine aortic endothelial (BAE) cells were scraped with a razor blade to create a denuded area on the culture dish. The number of endothelial cells that moved from the edge of the wound into the denuded area in the presence of varying concentrations of oligonucleotide ligands was determined after 8 hours. The movement of BAEs under untreated conditions is dependent on endogenous bFGF and can be inhibited by addition of neutralizing antibodies to bFGF. Ligand 2IA-ts (5'-cucggcgaaacgccccggggc-3' (SEQ ID NO:44)) inhibited BAE migration in a dose dependent manner at concentrations greater than 50 nM (Ligand 2IA-ts is a chemically synthesized analogue of 2'-NH<sub>2</sub> Ligand 2IA-t (SEQ ID NO:186) in which the terminal 2'-aminoxyridine has been converted to deoxyxycytidine. This substitution does not affect high affinity binding to bFGF). The control ligand deoxy(2IA-ts) (all deoxy sequence equivalent of 2IA-t: 5'-gcgtccggaaacgccccggggc-3' (SEQ ID NO:45)) did not inhibit BAE migration at the same concentrations. In fact a moderate stimulation of migration was observed. The extent of inhibition at high RNA ligand concentrations varied significantly between experiments ranging from almost 100% to < 50% inhibition (data not shown). This is probably related in part to variable expression of other motility-inducing growth factors by BAE cells between

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experiments as well as subtle differences in the state of the cells at the time of wounding. Importantly, the total amount of motility that could be inhibited by 21A<sub>ts</sub> at high concentrations was comparable in all experiments to the effect of 100 µg/ml neutralizing bFGF antibody. This concentration of antibody is generally sufficient to inhibit all of the bFGF-dependent migration of endothelial cells. In a separate experiment we established that the oligonucleotides used in this experiment are not appreciably degraded over the duration of this experiment (8 hr) in a variety of cell culture conditions (data not shown).

## 15 EXAMPLE 8. bFGF DNA LIGANDS.

The SELEX protocol was performed in a manner similar to that described in Example 1 to obtain single stranded DNA (ssDNA) ligands to bFGF.

Here, SELEX is performed with single stranded DNA (ssDNA) starting with the three separate sets of synthetic DNA oligonucleotide templates and primers (Experiments 1-3) shown in Table XIX. These experiments are further split into two different methods of ssDNA partitioning from double stranded DNA (dsDNA). Briefly, in Experiment 1 a population of synthetic DNA oligonucleotides (40N2, SEQ ID NO:321) containing 40 random nucleotides flanked by invariant primer annealing sites was amplified by the Polymerase Chain Reaction (PCR) using oligos 3p2 (SEQ ID NO:323) and <sup>32</sup>P end labeled 5p2 (SEQ ID NO:321) as primers. Oligo 3p2 has three biotin phosphoramidites covalently attached to its 5' terminus during synthesis. In order to generate the ssDNA library from the PCR products, oligo 40N2 was separated from its complement. This was achieved by incubating the PCR reaction in the presence of a 10 fold molar excess of Pierce streptavidin over the biotinylated complement strand. The non-biotinylated ssDNA 40N2 was

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then purified away from the streptavidin labeled complement strand on a 12% denaturing gel. The ssDNA was eluted from the gel and precipitated, and the ssDNA library used for the selections.

Experiments 2 and 3 used two different populations of synthetic DNA oligonucleotides, oligos 40NBH1 (SEQ ID NO:325), and 30N7.1PS (SEQ ID NO:328), containing 40 and 30 random nucleotides respectively flanked by invariant primer annealing sites. The DNA pools were amplified by the Polymerase Chain Reaction (PCR) using oligos 3pBHI (SEQ ID NO:325) and 30N7.1PS (SEQ ID NO:328) and 5p7.1PS (SEQ ID NO:327) in Experiment 3 as primers for the appropriate invariant regions on template molecules. Oligos 3pBHI and 3p7.1PS had two biotin molecules and two additional A nucleotides covalently attached via standard phosphoramidite coupling to their 5' terminus during synthesis. The non-biotinylated primer was end labeled with <sup>32</sup>P. The radiolabeled non-biotinylated single-stranded PCR products were size-purified away from the biotinylated strand on 8% denaturing acrylamide gels to give single stranded degenerate DNA pools. DNA templates for PCR and the corresponding primers were all synthesized chemically (Operon). The random region was generated by utilizing an equimolar mixture of the four nucleotides during oligonucleotide synthesis. Using the above methods, three pools of ssDNA oligonucleotides were created that contain internal random regions. From each starting ligand pool approximately 10<sup>14</sup> molecules of DNA was incubated with bFGF at an excess of DNA to target. Oligonucleotides bound to bFGF can be effectively selected from the unbound species by filtration through nitrocellulose membrane filters. The nitrocellulose filters (Millipore, 0.45 µm pore size, type HA) were secured on a

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filter manifold pre-washed with PBS, the incubation mix washed through and the filter washed with 0.5 M Urea and PBS buffer to remove non-specific DNA from the filter.

The selected DNA (which typically amounts to 1-5% of the total input DNA) was then extracted from the filters. Amplification of the selected ssDNA was performed by PCR under standard conditions yielded sufficient amounts of double-stranded DNA for the next round of selection.

10 Selections were performed at a large molar excess of ssDNA over protein to promote competition among DNA ligands for the limited number of available target binding sites. The percent of target-dependent DNA retention was minimized for each selection to ensure maximum enrichment of the library for target binders; however, to avoid propagation of members with high affinity for nitrocellulose, selections in which target-free (background) retention was greater than 10% of target-dependent retention were repeated. Target-free

15 selections were performed to measure and correct for background binding levels. The fraction of total DNA retained by the filters was calculated by measuring radiation without fluor in a scintillation counter. The affinity of the pool for bFGF was measured periodically throughout each of the three selection experiments. As the affinity of the population for bFGF increased, the concentrations of ligand and target were reduced accordingly, while the ligand was maintained at an excess concentration, to increase selection stringency. Table

20 XX shows a typical SELEX progression as was seen in Experiment 3. The nucleic acid concentration was maintained at a five fold excess to the bFGF concentration, in all but the first round. Attempts were made to maintain a level of background that was 10 fold lower than the percent bound. The binding affinity was

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tested after round 0, 8, 10 and 11 to follow the progression.

#### Cloning and Sequencing

As indicated in Table XX, significant improvement in affinity of DNA ligands to bFGF was observed in each of the three experiments after ten rounds of selection. Individual members of these enriched pools were then cloned into Stratagene PCR Script SK (+) or pUC18 vector and sequenced. Sequencing of the isolates resulted in 36 clones. Experiment 2 resulted in 29, and Experiment 3 resulted in 43. As shown in Table XXI, five distinct families could be identified based on 40% or better overlap in sequence homology. A number of sequences with no obvious homology to members of the five families were also present. These sequences are listed as orphans. Each family is further divided into the three different SBELEX experiments. The consensus sequence for Family 1 ligands is defined by a contiguous stretch of 9 bases, GGGCTTGCAAN (SEQ ID NO:340) where the two N positions are covariant combination of all four bases. This suggests a minimal structure consisting of a 4 nucleotide loop that includes the strongly conserved GCA sequence. The loop is closed by the formation of a stem containing a T-A basepair and the covariant base pair position.

Determination of Binding Affinities for bFGF  
Equilibrium Dissociation Constant.  
In the simplest case, equilibrium binding of DNA to bFGF can be described by equation 3:

$$\text{DNA} \bullet \text{bFGF} \rightleftharpoons \text{DNA} + \text{bFGF} \quad (3)$$

The fraction of bound DNA ( $d$ ) is related to the concentration of free protein,  $[P]$ . Where the

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concentration of free protein approximates the concentration of total protein (equation 4):

$$q = f[P] / ([P] + K_d) \quad (4)$$

5

where  $K_d$  is the equilibrium dissociation constant and  $f$  reflects the efficiency of retention of the protein-DNA complexes on nitrocellulose filters. Mean value of  $f$  for bFGF was determined to be 0.82.

10

In order to eliminate higher order structures, all DNA solutions were heated to 90 °C in PBS for 2-3 minutes and cooled on ice prior to incubation with protein. Relative binding affinity of individual ligands to bFGF cannot be predicted from sequence information. The

15

majority of sequence isolates were therefore screened for their ability to bind to bFGF by measuring the fraction of radiolabeled DNA bound to nitrocellulose filters following incubation with 1 nM protein. This screening method was sufficient to discern those isolates with superior binding to bFGF. Binding of these select isolates was then analyzed in more detail.

High-affinity DNA ligands for bFGF were found in all five sequence families (see (\*) in Table XXI), but the DNAs with the lowest  $K_d$  values (i.e. ligands with highest affinity) were found in family 1.

The isolates tested for affinity for bFGF are listed in Table XXII.

#### Truncation Analysis.

Removal of nucleotides non-essential for binding was performed on selected ligands with high affinity for bFGF.  $K_d$ s below 1 nM. Those ligands are M225, M19, m234, M235, and D12 (SEQ ID NO:359, 353, 387, 360, 332). The minimum size of the region necessary for binding was determined to be 35 bases for M225, M19 and D12 (See Truncations, Table XXI M225t3 (SEQ ID NO:364), M19t2 (SEQ

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ID NO:365), D12t2 (SEQ ID NO:341)). The ligand with the smallest essential sequence, m234, was isolated from Family 2, Experiment 3 and contains 24 nucleotides (m234t2 (SEQ ID NO:391)). The truncated ligands were

tested for binding to bFGF. After truncation, ligands M225t3, M19t2, D12t2, M235t2, and m234t2 have  $K_d$  values of 0.7 nM, 1 nM, 1 nM, 1 nM, and 6 nM respectively (Table XXII). All five of the truncated molecules lost some of their affinity for bFGF in comparison to the full length ligands. The binding affinity is regained when an additional G-C base pair is added to the blunt end stem of M225t3. This molecule is termed M225t3GC (SEQ ID NO:443). The binding of M225t3GC is 0.2 nM compared to 0.7 nM for M225t3 without the additional base pair (Table XXII).

#### Receptor Binding Studies.

The truncated molecules were tested for their ability to inhibit binding of bFGF to its low- and the high-affinity cell-surface receptors.

20

bFGF labeled with  $^{125}$ I was purchased from Amersham. Confluent baby hamster kidney (BHK) cells were washed extensively with PBS and then incubated for 2 hours at 4 °C with a MEM medium containing 10 ng/ml  $^{125}$ I-bFGF in PBS, 0.1% HSA, 1 unit/ml RNasin, and serial dilutions of high-affinity DNA. The amount of  $^{125}$ I-bFGF bound to the low- and the high-affinity receptor sites was determined as described by Moccatelli (1987) *et al.*

All five ligands competed for the low-affinity and high-affinity receptor sites while the unselected (random) RNA did not. All five ligands show inhibition in the nanomolar range.

#### Specificity.

Ligand M225t3 (SEQ ID NO:364) the truncated version of the full length isolate M225 (SEQ ID NO:359) was chosen as the preferred ligand for further study. This

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was based on its sub-nanomolar binding (Table XXII), its  $T_m$  of 68 °C which indicates a stable structure, possibly containing a G-C rich stem, and a 35 base truncation. The sequence of M225t3 results in a DNA that folds into a structure containing a 6 base G-C stem terminating in a blunt end. Using the covariant site in the conserved region, a GYK loop can be proposed in the consensus region.

In order to address the question of specificity, ligand M225t3 was tested for binding to vascular endothelial growth factor and human chorionic gonadotropin, both heparin-binding proteins. It was found that the affinity of M225t3 for these proteins was relatively weak ( $K_d > 0.2 \mu\text{M}$ ).

**EXAMPLE 9. CONJUGATION OF bFGF LIGAND TO PEG.**

In an effort to determine whether enhanced circulation time could be obtained by conjugating the bFGF to a high molecular weight species, such as PEG, M225t3 DNA was synthesized with a 3' carbon linker terminating in a primary NH<sub>2</sub> group. The modified DNA was then reacted with an excess of an N-hydroxysuccinimidyl active ester of PEG 3400. The product was isolated as a slower running band on a gel. It was then labeled and a binding assay performed. The PEG modified M225t3 binds with a similar affinity to bFGF as the non modified ligand. The PEG modified M225t3 binds with a  $K_d$  of 1 nM.

**EXAMPLE 10. EVOLUTION OF HIGH AFFINITY RNA LIGANDS TO THROMBIN.**

High affinity RNA ligands for thrombin were isolated by SELEX, as generally described in Example 1. Briefly, random RNA molecules used for the initial candidate mixture were generated by *in vitro* transcription from a 102 nucleotide double-stranded DNA template containing a

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random cassette 30 nucleotides (30N) long. A population of 1.0<sup>11</sup> 30N DNA templates were created by PCR, using a 5' primer containing the T7 promoter for *in vitro* transcription, and restriction sites in both the 5' and 3' primers for cloning. SELEX was performed with an RNA candidate mixture containing the following 76 nucleotide sequences: 5'-AGAUUCCGUU CAGGAGCGC(30N)GACGUA CAGCUCUUCGUACGCG-3' (SEQ ID NO:320).

The RNA concentration for each round of SELEX was approximately 2-4 X 10<sup>-3</sup> M and concentrations of thrombin (Sigma, 1000 units) went from 1.0 X 10<sup>-4</sup> in the 1st round to 4-8 X 10<sup>-7</sup> in rounds 2 and 3 and 2.4 X 10<sup>-7</sup> in rounds 4-12. The binding buffer for the RNA and protein was 100 mM NaCl, 50 mM Tris-Cl, pH 7.7, 1 mM DTT, and 1 mM MgCl<sub>2</sub>. Binding was for 5 minutes at 37°C in a total volume of 100  $\mu\text{l}$  in rounds 1-7 and 200  $\mu\text{l}$  in rounds 8-12. Each binding reaction was filtered through a pre-wetted (with 50 mM Tris-Cl, pH 7.7) nitrocellulose filter (2.5 cm Millipore, 0.45  $\mu\text{m}$ ) in a Millipore filter binding apparatus, and immediately rinsed with 5 ml of the same buffer. The RNA was eluted from the filters in 400  $\mu\text{l}$  phenol (equilibrated with 0.1 M NaOAc pH 5.2), 200  $\mu\text{l}$  freshly prepared 7 M urea as described (Tuerk et al. (1990) J. Mol. Biol. 213:749-761). The RNA was precipitated with 20  $\mu\text{g}$  tRNA, and was used as a template for cDNA synthesis, followed by PCR and *in vitro* transcription to prepare RNA for the subsequent round. The RNA was radio-labeled with <sup>32</sup>P-ATP in rounds 1-8 so that binding could be monitored. In order to expedite the time for each round of SELEX, the RNA was not labeled for rounds 9-12. RNA was prefiltered through nitrocellulose filters (1.3 cm Millipore, 0.45  $\mu\text{m}$ ) before the 3rd, 4th, 5th, 8th, 11th, and 12th rounds to eliminate selection for any nonspecific nitrocellulose binding.

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Binding curves were performed after the 5th, 8th, and 11th rounds to estimate changes in  $K_d$  of the bulk RNA (data not shown). These experiments were done in protein excess at concentrations from  $1.2 \times 10^{-4}$  to  $2.4 \times 10^{-3}$  M at a final RNA concentration of  $2 \times 10^{-9}$  M. The RNA for these binding curves was labeled to high specific activity with  $^{32}P$ -NTP or  $^{32}P$ -UTP. Binding to nitrocellulose filters was as described for the rounds of SELEX, except that the filter bound RNA was dried and counted directly on the filters.

**EXAMPLE 11. CLONING AND RNA SEQUENCING.**

RNA recovered from the 12th round of SELEX was reverse transcribed into DNA with AMV reverse transcriptase (Life Sciences, Inc.) and the resulting DNA was amplified by PCR using the  $^{32}P$  5' end-labeled 3' complementary PCR primer. Digestion at restriction enzyme sites in the 5' and 3' fixed regions were used to remove the 3'N region which was subsequently ligated into the complementary sites in the *E. coli* cloning vector pUC18. Ligated plasmid DNA was transformed into JM103 cells and screened by blue/white colony formation. Colonies containing unique sequences were grown up and miniprep DNA was prepared. Double-stranded plasmid DNA was used for dideoxy sequencing with the Sequenase kit version 2.0 and  $^{35}S$ -dATP (Amersham). Twenty eight individual clones were sequenced (see Table XIII). The ligands were grouped into two classes based upon primary sequence homology.

**EXAMPLE 12. DETERMINATION OF 5' AND 3' BOUNDARIES.**

In order to identify the minimal sequence requirements for high affinity binding, 5' and 3' boundary experiments were performed with end-labeled RNA.

Prior to end-labeling, RNA transcribed with T7 polymerase

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was gel purified by UV shadowing. The RNA was 5' end-labeled by dephosphorylating the 5' end with alkaline phosphatase 1 unit, for 30 minutes at 37 °C. Alkaline phosphatase activity was destroyed by phenol:chloroform extraction. RNA was subsequently end-labeled with  $\gamma^{32}P$ -ATP in a reaction with polynucleotide kinase for 30 minutes at 37 °C.

RNA was 3' end-labeled with (5'- $^{32}P$ )dGMP and RNA ligase, for 30 minutes at 37 °C. 5' and 3' end-labeled RNAs were gel band purified on an 8%, 8 M urea, polyacrylamide gel.

2 pmole RNA 3' or 5' end-labeled for the 5' or 3' boundary experiments, respectively were hydrolyzed in 50 mM Na<sub>2</sub>CO<sub>3</sub> (pH 9.0) and 1 mM EDTA in a 10  $\mu$ l reaction for 10 minutes at 37 °C. The reaction was stopped by adding 1/5 volume 3 M NaOAc (pH 5.2), and freezing at -20 °C. Binding reactions were done at 3 protein concentrations, 40 nM, 10 nM and 2.5 nM, in 3 volumes (100  $\mu$ l, 400  $\mu$ l, and 1600  $\mu$ l, such that the amount of protein was kept constant) containing 1X binding buffer and 2 pmoles RNA. Reactions were incubated for 10 minutes at 37°C, filtered through a pre-wet nitrocellulose membrane, and rinsed with 5 ml wash buffer. The RNA was eluted from the filters by dicing the filter and shaking it in 200  $\mu$ l 7 M urea and 400  $\mu$ l phenol (pH 8.0) for 15 minutes at 20 °C. After adding 200  $\mu$ l H<sub>2</sub>O, the phases were separated and the aqueous phase extracted once with chloroform. The RNA was precipitated with 1/5 volume 3 M NaOAc, 20  $\mu$ g carrier tRNA, and 2.5 volumes ethanol. The pellet was washed once with 70% ethanol, dried, and resuspended in 5  $\mu$ l H<sub>2</sub>O and 5  $\mu$ l formamide loading dye. The remainder of the alkaline hydrolysis reaction was diluted 1:10 and an equal volume of loading dye was added.

To locate where on the sequence ladder the boundary existed, an RNase T1 digest of the ligand was

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electrophoresed alongside the alkaline hydrolysis reaction and binding reactions. The digest was done in a 10  $\mu$ l reaction containing 500 fmoles end-labeled RNA and 10 units RNase T1 in 7 M urea, 20 mM sodium citrate (pH 5.0) and 1 mM EDTA. The RNA was incubated for 10 minutes at 50 °C without enzyme and then another 10 minutes after adding enzyme. The reaction was slowed by adding 10  $\mu$ l loading dyes and incubating at 4 °C. Immediately after digestion, 5  $\mu$ l of each of the digest, hydrolys, and 3 binding reactions were electrophoresed on a 12% sequencing gel. The boundary experiments gave the boundaries depicted in Table XIII. Based upon these boundaries, possible secondary structures of the thrombin ligand are shown in Figure 7.

**EXAMPLE 13. SYNTHESIS OF RNA.**

RNA molecules corresponding to lower limits of nucleotide sequence required for high affinity binding to thrombin as determined by the boundary experiments (Table XII and Figure 7) were synthesized on an Applied Biosystems 394 DNA/RNA Synthesizer. These RNA molecules include the Class I clone 16 (SEQ ID NO:212) hairpin structures of 24 nucleotides (24R) and 39 nucleotides (39R) and the Class II clone 27 (SEQ ID NO:214) hairpin of 33 nucleotides (33R).

**EXAMPLE 14. IN VITRO TRANSCRIPTION AND BINDING OF 2'-NH<sub>2</sub>-MODIFIED AND UNMODIFIED RNA LIGANDS.**

Four DNA plasmids with unique 3'ON sequences were chosen for in vitro transcription of selected unmodified and 2'-NH<sub>2</sub> modified RNA ligands from Class I and Class II. 2'-NH<sub>2</sub> modified RNA was transcribed directly from the pUC18 plasmid miniprep dsDNA template with T7 RNA polymerase in a reaction containing ATP, GTP, 2'-NH<sub>2</sub>-UTP and 2'-NH<sub>2</sub>-CTP. Unmodified RNAs were transcribed in a

mixture containing ATP, GTP, UTP, and CTP. For <sup>32</sup>P-labeled RNA, <sup>32</sup>P-ATP was included in the reaction.

labelled RNA was transcribed with conventional nucleotides, as well as, with the 2'-NH<sub>2</sub> derivatives of CTP and UTP. Binding curves with these individual RNAs were established using the binding buffer and thrombin (1000 units, Sigma) concentrations from  $1.0 \times 10^{-6}$  to  $1.0 \times 10^{-10}$  M. Human  $\alpha$  thrombin (Enzyme Research Laboratories, ERL) was also used to determine binding affinities of RNA at concentrations from  $1.0 \times 10^{-6}$  to  $1.0 \times 10^{-10}$  M.

The 2'-NH<sub>2</sub>-CTP/UTP modified RNAs of Class I and Class II showed a significant drop in binding when compared to the unmodified RNA (Figure 9). Binding by the bulk 3'ON RNA, however, showed a slight increase in affinity when it was modified.

Binding of the 5' end-labeled single stranded 15mer DNA 5'-GTTGGTGTGGTG-3' (G15D) (SEQ ID NO:189) described by Book et al. (1992) Nature 315:564-565, was determined under the binding conditions described herein with ERU thrombin and compared to binding by the radiolabelled RNA hairpin structures described above. (see Figure 8C).

**EXAMPLE 15. COMPETITION EXPERIMENTS.**

To determine whether the RNA ligands described can compete for binding of the DNA 15mer G15D to thrombin, equimolar concentrations (1  $\mu$ M) of thrombin and the 5' end labeled DNA 15mer G15D were incubated under filter binding conditions ( $K_d$  of approximately 200 nM) in the presence and absence of 'cold' unlabeled RNA or DNA ligand at varying concentrations from 10 nM to 1  $\mu$ M. In the absence of competition, RNA binding was 30%. The protein was added last so competition for binding could occur. The RNA ligands tested for competition were the

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(Table XIV).

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Class I clone 16 (SEQ ID NO:212) synthetic RNAs 24mer (24R) and 39mer hairpins (39R) and the Class II 27 (SEQ ID NO:214) synthetic RNA 33mer (33R). Results are expressed as the relative fraction of G15 bound (G15 with competitor/G15 without competitor) versus the concentration of cold competitor.

To determine whether Class I RNAs can compete for binding with Class II RNAs and to confirm the competition with the G15 DNA, equimolar concentrations (300 nM) of thrombin and the 5' end-labelled Class II RNA 33 hairpin were incubated under filter binding conditions in the presence or absence of 'cold' unlabelled RNA 24 or DNA G15D at varying concentrations from 100 nM to 32  $\mu$ M.

Results are expressed as the relative fraction of RNA 33 bound (RNA 33 with competitor/RNA 33 without competitor) versus the concentration of cold competitor (Figure 10).

**EXAMPLE 16. CHROMOGENIC ASSAY FOR THROMBIN ACTIVITY AND INHIBITION BY RNA LIGANDS.**

The hydrolysis by thrombin of the chromogenic substrate S-2238 (H-D-Phe-Pip-Arg-pNitroaniline [H-D-Phe-Pip-Arg-pNA]) (Kabi Pharma) was measured photometrically at 405 nm due to the release of p-nitroaniline (pNA) from the substrate.

20 H-D-Phe-Pip-Arg-pNA + H<sub>2</sub>O  $\xrightarrow{\text{Thrombin}}$   
 H-D-Phe-Pip-Arg-OH + pNA

25

30 Thrombin was added to a final concentration of 10<sup>-6</sup> or 10<sup>-7</sup> M to a reaction buffer (50 mM sodium citrate, pH 6.5, 150 mM NaCl, 0.1% PEG), containing 250  $\mu$ M S2238 substrate at 37 °C. For inhibition assays, thrombin plus RNA (equimolar or at 10-fold excess) were preincubated 30 secs at 37 °C before adding to the reaction mixture

EXAMPLE 17. FIBRINOGEN CLOTTING.

Thrombin was added for a final concentration of 2.5 nM to 400  $\mu$ L incubation buffer (20 mM Tris-acetate, pH 7.4, 140 mM NaCl, 5 mM KCl, 1 mM CaCl<sub>2</sub>, 1 mM MgCl<sub>2</sub>) containing 0.25 mg/ml fibrinogen and 1 U/L RNase inhibitor (RNasin, Promega) with or without 30 nM RNA Class I or 60 nM RNA Class II at 37 °C. Time in seconds from addition of thrombin to clot formation was measured by the tilt test (Table XIV).

**EXAMPLE 18. SPECIFICITY OF THROMBIN BINDING.**

The binding affinity of the full-length class I RNA 16 (SEQ ID NO:198), class II RNA 27 (SEQ ID NO:209) and bulk 30nT RNA for the serum proteins Antithrombin III (ATIII) and Prothrombin was determined by filter binding, as described above for the evolution of high affinity RNA ligands (Example 10). These experiments were done in protein excess at concentrations from 1  $\times$  10<sup>-9</sup> to 5  $\times$  10<sup>-10</sup> M at a final RNA concentration of 2  $\times$  10<sup>-9</sup> M (Figure 11).

**EXAMPLE 19. EVOLUTION OF HIGH AFFINITY DNA LIGANDS TO THROMBIN.**

25 High affinity single-stranded DNA (ssDNA) ligands for thrombin were isolated by SELEX. Two populations of approximately 10<sup>14</sup> ssDNA molecules with either a 30-nucleotide (30N) (SEQ ID NO:215) or 60-nucleotide (60N) (SEQ ID NO:260) variable region and 5' and 3' fixed regions were synthesized for the initial selection. Thrombin and DNA were incubated in a buffer containing 50 mM Tris-Cl, pH 7.5, 100 mM NaCl, 1 mM MgCl<sub>2</sub>, at 37 °C for 5 minutes. The thrombin-bound DNA was partitioned from unbound DNA by nitrocellulose-filter binding. DNA was eluted from the filters by denaturation and

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phenol/chloroform extraction. A double-stranded DNA product with 3 biotin molecules at the 5' end of the complementary strand was created and amplified by PCR using a 3' complimentary biotinylated primer and sense 5' primer. The double-stranded product was bound to a streptavidin-agarose matrix and the nonbiotinylated ssDNA template was isolated by alkaline denaturation. This ssDNA template pool was used for the following round of SELEX.

Nitrocellulose filter binding was used to determine K<sub>d</sub>s. No additional improvement in binding was seen after 12 rounds of SELEX where the K<sub>d</sub>s for the 3ON and 6ON populations were both determined to be approximately 8 nM (Figure 12). The K<sub>d</sub>s for the bulk 3ON and 6ON populations after 12 rounds of SELEX were approximately 8 μM and 5 μM, respectively. Double-stranded DNA from the 12th round was digested with restriction enzyme sites in the 5' and 3' fixed regions and ligated into the complementary sites of the *E. coli* cloning vector pUC18.

Plasmid DNA was prepared and used for dideoxy sequencing by PCR. Twenty-eight clones from the 3ON population were sequenced and 24 unique sequences were identified while thirty-two clones from 6ON population were sequenced and 31 unique sequences were identified (Table XVI). ssDNA from individual clones 6 (SEQ ID NO:219), 8 (SEQ ID NO:221), 14 (SEQ ID NO:224), 16 (SEQ ID NO:226), and 35 (SEQ ID NO:238) from the 3ON population and 7 (SEQ ID NO:236), 18 (SEQ ID NO:256), and 27 (SEQ ID NO:264) from the 6ON population was prepared and K<sub>d</sub>s were determined by nitrocellulose filter binding. K<sub>d</sub>s ranged from 0.4 nM to 9.4 nM for the 3ON DNAs and from 0.9 to 2.5 nM for the 6ON DNAs (Table XVI). Regions of homology between these DNA are indicated in bold and G-nucleotide residues that may be involved in quadruplex formation are also underlined. A truncated ligand of 38 nucleotides from

the high affinity clone 60-18 (SEQ ID NO:278) (K<sub>d</sub>=0.9 nM), designated 60-18(38) (SEQ ID NO:279) has been identified (K<sub>d</sub>=1.9 nM; Table XVI) that retains high-affinity binding (Figure 13) and inhibits clotting (Figure 14).

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**TABLE I. OLIGONUCLEOTIDES USED IN SELEX EXPERIMENTS A AND B TO SELECT RNA LIGANDS TO bFGF.**

EXPERIMENT A	SEQUENCE 5'-3'	SEQ ID NO.
Starting RNA	GGGAGCUCAGAAUAAACGCUCAAANNNNNNNNNNNNNN NNNNNNNNNNNNNNNUUCGACAUGAGGCCGGAUCCGGC	SEQ ID NO:1
PCR Primer 1	<u>HindIII</u> CCGAAGCTTAATACGACTCACTATAAGGGAG T7 Promoter CTCAGAATAAACGCTCAA	SEQ ID NO:2
PCR Primer 2	<u>BamH1</u> GCCGGATCCGGGCCTCATGTCGAA	SEQ ID NO:3
<b>EXPERIMENT B</b>		
Starting RNA	GGGAGAUGCUGUCGAGCAUGCUGNNNNNNNNNNNNNN NNNNNNNNNNNNNNNGUAGCUAACAGCUUUGUCGACGGG	SEQ ID NO:4
PCR Primer 1	<u>HindIII</u> CCCGAAGCTTAATACGACTCACTATAAGGGAG T7 Promoter ATGCCTGTCGAGCATGCTG	SEQ ID NO:5
PCR Primer 2	<u>Sall</u> CCCGTCGACAAAGCTTTAGCTAC	SEQ ID NO:6

HEXAGEN/FIGURES/TABLE I-EAH

**TABLE II. FAMILY 1 SEQUENCES FROM SELEX EXPERIMENTS A AND B.**

**CONSENSUS SEQUENCE**  
**CUAACCAGG (SEQ ID NO:7)**

gggagcucagaaaaacgcucaa-[30N]-uucgacaugggccggauccggc (SEQ ID NO:1)

FAMILY 1	CLONE (30N)	SEQ ID NO.
4A	UGCUAUUCGCCUAACUCGGCGCUCCUACCU	SEQ ID NO:8
5A	AUCUCCUCCCCGUCAAGCUAACCUUGGCCAC	SEQ ID NO:9
7A	UCGGCGAGCUAACCAAGACACACUCGCGUGAC	SEQ ID NO:10
10A	GUAGCACUAUCGGCCUAACCCGGUAGCUCC	SEQ ID NO:11
13A	ACCCGCGGCCUCCGAAGCUAACCAAGGACAC	SEQ ID NO:12
14A	UGGGUGCUAACCAAGGACACACCCACCGCUGU	SEQ ID NO:13
16A	ACGCACAGCUAACCAAGGCCACUGUGCCCC	SEQ ID NO:14
18A	CUGCGUGGUUAACCAAGACUGCCUGGGCGA	SEQ ID NO:15
21A	UGGGUGCUAACCAAGGCCACACCCUGCGU	SEQ ID NO:16
25A	CUAGGUGCUAUCCAGGACACUCUCCCUGGUCC	SEQ ID NO:17
29A	UGCUAUUCGCCUAAGCUCGGCGCUCCUACCU	SEQ ID NO:18
38A	AGCUAUUCGCCAACCCGGCGCUCCGACCC	SEQ ID NO:19
39A	ACCAAGCUGCGUGCAACCGCACAUGCCUGG	SEQ ID NO:20
56A	CAGGCCCCGUCGUAAAGCUAACCUUGGACCCU	SEQ ID NO:21
61A	UGGGUGCUAACCAAGGCCACACACUCACGCUGU	SEQ ID NO:22

HEXAGEN/FIGURES/TABLE II-EAH

TABLE III. FAMILY 2 SEQUENCES FROM SELEX EXPERIMENTS A AND B.

CONSENSUS SEQUENCE:  
**RRGGHAACGYWNNGDCAAGNNCACYY**  
 (SEQ ID NO:23)

gggagcucagaauaaacgcucaa-[30N]-uucgacaugaggccggauccggc (SEQ ID NO:1)

FAMILY 2	CLONE (30N)	SEQ ID NO.
11A	GGGUAAACGUUGU	SEQ ID NO:24
12A	GGGGCAACGCUACA	SEQ ID NO:25
26A	CGUCAGAAGGCAACGUUA	SEQ ID NO:26
27A	CCUCUCGAAGACAACGCUGU	SEQ ID NO:27
47A	AGUGGGAAACGCUACUUGACAAG	SEQ ID NO:28
65A	GGCUACGCUAAU	SEQ ID NO:29

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gggagaugccugucgagcaugcug-[30N]-guagcuuacacgcuuuggucgacggg (SEQ ID NO:4)

FAMILY 2	CLONE (30N)	SEQ ID NO.
1B	CUCUGGUAAACGCAAU	SEQ ID NO:30
2B	AGCCGCAGGUAAACGGACC	SEQ ID NO:31
6B	ACGAGCUUCGUAACGCUAUC	SEQ ID NO:32
8B	AAGGGGAAACGUUGA	SEQ ID NO:33
9B	AGGUAACGUACU	SEQ ID NO:34

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TABLE III. (CONTINUED)

11B	GAGGUAAACGUAC	GACAAGACCACUCCAACU	SEQ ID NO:35
12B	AGGUAAACGCUA	GUCAAGUGGCACUCGACAU	SEQ ID NO:36
13B	GGGAAACGCUAUC	GACGAGUGGCCACCGGCA	SEQ ID NO:37
14B	CCGAGGGUAACGUUGG	GUCAAGCACACCUUC	SEQ ID NO:38
15B	UCGGGGUAAACGUUU	GGCAAGGC ACCCGAC	SEQ ID NO:39
19B	GGUAAACGCUUG	GACAAGUGGCACCAAGCUGC	SEQ ID NO:40
22B	AGGGUAACGUACU	GGCAAGCUCACCUUCAGC	SEQ ID NO:41
28B	AGGGUAACGUUA	GUCAAGAC ACCUCAAGU	SEQ ID NO:42
29B	GGGUAAACGCAUU	GGCAAGAC ACCCAGCCCC	SEQ ID NO:43
36B	GAGGAAACGUACC	GUCCAGGCC ACUCCAUGC	SEQ ID NO:44
38B	AGGUAAACGCUA	GUCAAGUGGCACUCGACAU	SEQ ID NO:45
48B	GGGUAAACGUGU	GACAAGAUACCCAGUUUG	SEQ ID NO:46
49B	CACAGGGCAACGCUUGCU	GACAAGUGGCACCU	SEQ ID NO:47

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TABLE IV. OTHER SEQUENCES FROM SELEX EXPERIMENTS A AND B.

gggagcucagauaaacgcucnn-[30N]-uucgacaugaggcccggauccggc (SEQ ID NO:1)

NUMBER	CLONE (30N)	SEQ ID NO.
8A	ACGCCAACGAGAGUCAGCAACAGAGCGUCCG	SEQ ID NO:48
9A	CCAGUGAGUCUGGUUAUCCCAUCGGCU	SEQ ID NO:49
24A	CUUCAGAACGGCAUAGUGGUUCGGCCGCGCC	SEQ ID NO:50
33A	AGGUACACUGCGUCACCGUACAUGCCUGGCC	SEQ ID NO:51
34A	UCCAACGAACGGCCCCUUCGUAUUCAGGCCACC	SEQ ID NO:52
36A	ACUGGAACCUGACGUAGUACAGCGACCCUC	SEQ ID NO:53
37A	UCUCGCUGCGCCUACACGGCAUGCCGGGA	SEQ ID NO:54
40A	GAUCACUGCGCAAUGCUGCAUACCCUGGUC	SEQ ID NO:55
43A	UCUCGCUGCGCCUACACGGCAUGCCGGGA	SEQ ID NO:56
44A	UGACCAGCUGCAUCCGACGAUUAUACCCUGG	SEQ ID NO:57
45A	GGCACACCUCAACGAGGUACGUUACGGCG	SEQ ID NO:58
55A	AGCGGAACGCCACGUAGUACGCCACCCUC	SEQ ID NO:59

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TABLE IV. (CONTINUED)

gggagaugecugucgagcaugcug-[30N]-guagcuuacagcuuuggucgacggg (SEQ ID NO:4)

NUMBER	CLONE (30N)	SEQ ID NO.
4B	ACCCACGCCGACAACCGAUGAGUUUCUCGG	SEQ ID NO:60
5B	UGCUUUGAAGUCCUCUCCCGCCUCUGAGGU	SEQ ID NO:61
7B	AUGCUGAGGAUAUUGUGACCAUCUUCGGGU	SEQ ID NO:62
16B	ACCCACGCCGACAACCGAUGAGCUCGG	SEQ ID NO:63
20B	AGUCCGGGAUGCCCCACUGGGACUACUUJU	SEQ ID NO:64
21B	AAGUCCGAAUGCACUGGGACUACCACUGA	SEQ ID NO:65
23B	ACUCUCACUGCGAUUCGAAAUCAUUGCCUGG	SEQ ID NO:66
40B	AGGCUGGGUCACCGACAACUGCCGCCAGC	SEQ ID NO:67
42B	AGCCGCAGGUACGGACCGGGCAGACCAUC	SEQ ID NO:68
26B	GCAUGAAGCGGAACUGUAGUACGCGAUCCA	SEQ ID NO:69

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TABLE V. REPEAT SEQUENCES FROM SELEX  
EXPERIMENTS A AND B.

ggggcucagaaacgcuaa-[30N]-uucgacauugaggccggauccggc (SEQ ID NO:1)

NUMBER	SEQ ID NO.	CLONE REPEATED
3A GGGUAACGUUUGACAAGUACACCUGCGUC	SEQ ID NO:70	11A
15A GGGUAACGUUUGACAAGUACACCUGCGUC	SEQ ID NO:71	11A
20A GGGUAACGUUUGACAAGUACACCUGCGUC	SEQ ID NO:72	11A
48A GGGUAACGUUUGACAACUACACCUGCGUC	SEQ ID NO:73	11A
58A GGGUAACGUUUGACAACUACACCUGCGUC	SEQ ID NO:74	11A
64A GGGUAACGUUUGACAACUACACCUGCGUC	SEQ ID NO:75	11A
28A CGUCAGAAGGCAACGUUAAGGAAGCACAC	SEQ ID NO:76	26A
30A GUAGCACUAUCGCCUAACCCGGUAGCUCC	SEQ ID NO:77	10A
23A ACCCGCGGCCUCCGAAGCUAACAGGACAC	SEQ ID NO:78	13A
46A AGGUACACUGCGUACCCGUACAUGCCUGGCC	SEQ ID NO:79	33A
49A AGGUACACUGCGUACCCGUACAUGCCUGGCC	SEQ ID NO:80	33A
50A GGCACACCUAACGAGGUACGUUACGGCG	SEQ ID NO:81	45A
41A GGGGCAACGUACAGACAAAGUGCACCCAAC	SEQ ID NO:82	12A
51A GGGGCAACGUACAGACAAAGUGCACCCAAC	SEQ ID NO:83	12A
54A GGGGCAACGUACAGACAAAGUGCACCCAAC	SEQ ID NO:84	12A
35A UGGGUGCUAACCGGACACACCCACGCUGU	SEQ ID NO:85	14A

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TABLE V. (CONTINUED)

gggagaugccugucgagcaugcug-[30N]-guagcuuacacgcuuuugucgacggg (SEQ ID NO:4)

NUMBER	SEQ ID NO.	CLONE REPEATED
18B CCGAGGGUAACGUUUGGUCAAGCACACCUC	SEQ ID NO:86	14B
24B GGGAAACGCUAUUCGACGAGUGCACCAGGCA	SEQ ID NO:87	13B
39B GGGAAACGCUAUUCGACGAGUGCACCAGGCA	SEQ ID NO:88	13B
37B ACUCUCACUGCGAUUCGAAAUCAUGCCUGG	SEQ ID NO:89	23B
43B GCAUGAAGCGGAACGUAGUACGCGAUCCA	SEQ ID NO:90	26B
46B GCAUGAAGCGGAACGUAGUACGCGAUCCA	SEQ ID NO:91	26B
25B AGGGUAACGUACUGGCAAGCUCACCUCAGC	SEQ ID NO:92	9B
33B AGGGUAACGUACUGGCAAGCUCACCUCAGC	SEQ ID NO:93	9B
31B GGUAACGCGUGGGACAAGUGCACCGCUGC	SEQ ID NO:94	19B

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TABLE VI. SECONDARY STRUCTURES AND DISSOCIATION CONSTANTS  
(K<sub>d</sub>) FOR A REPRESENTATIVE SET OF HIGH-AFFINITY  
LIGANDS FROM FAMILY 1.

LIGAND	STRUCTURE <sup>a</sup>	K <sub>d</sub> , nM	SEQ ID NO: (PARENT SEQUENCE)
5A-t <sup>b</sup>	CC AA CCUC GUCCAA---GCU C ggag cagcuu CGG C ua CAC U	23 ± 3	190
7A-t <sup>b</sup>	AA CGGCAG---CU C GUCCUC GA C ACA A	5.0 ± 0.5	191
13A-t <sup>b</sup>	C A CCG GCCCUC---CGAAG---CU A ggc-ccggag gcuuc GA C uaca ACAG C	3.2 ± 0.5	193

TABLE VI. (CONTINUED)

LIGAND	STRUCTURE <sup>a</sup>	K <sub>d</sub> , nM	SEQ ID NO: (PARENT SEQUENCE)
14A-t <sup>b</sup>	cucaa A aaacg UGGUG---CU A uuUGU- ACCCAC GA C CGC ACAG C	3.0 ± 0.5	194
21A-t <sup>b</sup>	A aaU---GGGU---CCUU A uUG CCCA CGGA C UCGU CAC C	8.1 ± 0.8	197
25A-t <sup>b</sup>	A CUA-GGUG---CU U GGU CCUC GA C C UCAG C	5.9 ± 1.4	198
39A-t <sup>b</sup>	CU A AACCAG GC--GUCA A uuGGUC--CG CACG C UA C	8.5 ± 1.2	201

<sup>a</sup>Strongly conserved positions are shown in boldface symbols. Nucleotides in the constant region are in lowercase type.

<sup>b</sup>The letter "t" is used to designate truncated sequences derived from the corresponding parent sequences (Figure XVII).

REAZEN/FIGURES/TABLE.6-EAM

TABLE VII. SECONDARY STRUCTURES AND DISSOCIATION CONSTANTS ( $K_d$ 's)  
FOR A REPRESENTATIVE SET OF HIGH-AFFINITY LIGANDS FROM FAMILY 2.

LIGAND	STRUCTURE*	$K_d$ , nM	SEQ ID NO: (PARENT SEQUENCE)
--------	------------	------------	---------------------------------

12A-t<sup>b</sup>                    CAACGCCU  
                               G      A  
                               C  
                               uc-aa---GGG      A  
                              ag uu    CCC      G  
                               c    CAA    A      A  
                               CGUGAAC

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26A-t<sup>b</sup>                    CAACGUUA  
                               A      G      U  
                               GUC GAAG      A  
                              cag-cuuC      G  
                               A      G  
                               CACGAAC

TABLE VII. (CONTINUED)

LIGAND	STRUCTURE*	$K_d$ , nM	SEQ ID NO: (PARENT SEQUENCE)
--------	------------	------------	---------------------------------

65A-t<sup>b</sup>                    CUACGUUA  
                               G      A  
                               A  
                               aacgcucaaG      U  
                              uuGUGGGGUUC      G  
                               A      A  
                               CGUGAAC

22B-t<sup>b</sup>                    UAACGUUA  
                               G      C  
                               agc-augcugAGG      U  
                              ucg ugCGACUCC      G  
                               a      A      G  
                               CUCGAAAC

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28B-t<sup>b</sup>                    UAACGUUA  
                               G      U  
                               augc-ugAGG  
                              ugUG ACUCC      A  
                               A      A      G  
                               CAGAACU

TABLE VII. (CONTINUED)

LIGAND	STRUCTURE*	K <sub>d</sub> , nM	SEQ ID NO: (PARENT SEQUENCE)
38B-t <sup>b</sup>	<pre>           UAACGCU           c   G   G gcaug ugAG      A ugUAC GCUC      G           A   A   U           CGUGAAC </pre>	4 ± 1	224
2B-t <sup>b</sup>	<pre>           UAACGCA           C   G   C AGC GCAG      C ucg ugUU      G           A   A   G           CCAGAGC </pre>	170 ± 80	210

\*Strongly conserved positions are shown in boldface symbols. Nucleotides in the constant region are in lowercase type.

<sup>b</sup>The letter "t" is used to designate truncated sequences derived from the corresponding patent sequences (Figure XVIII).

REAGEN/FIGURES/TABLE 7-EAM

TABLE VIII. 2'-NH<sub>2</sub> RNA LIGANDS TO bFGF.

5'-GGGAGACAAGAAUAACGUCAA [-30N-] UUCGACAGGAGGCUCACAACAGGC-3' (SEQ ID NO: 95)

5'-GGGAGGACGAUGCGG [-50N-] CAGACGACTCGCCCGA-3' (SEQ ID NO: 98)

FAMILY 1A		CORRESPONDING CLONE	SEQ ID NO:
14A	ACANGGAGUUGUGUGGAAGGCAGGGGGAGG	30N	101
15A	UGUGUGGAAGGCAGUGGGAGGUUCAGUGGU	30N	102
17A	AAAGUUGUGUGGAAGACAGUGGGAGGUGAA	30N	103
21A	GUAGACUUAUGUGUGGAAGACAGCGGGUGG	30N	104
29A	NNAGUUGUGUGGAAGACAGUGGGGGUUGA	30N	105
38A	GGUGUGUNGAAGACAGUGGGUNGUUAGNC	30N	106
49A	AUGGUGUGUGGAAGACAGUGGGUGGUUGCA	30N	107
54A	ACUGUUGUGUGGAAGACAGCGGGUGGUUGA	30N	108
60A	AAUGUAGGCUGUGUGGUAGACAGUGGGUGG	30N	109
68A	GAUGUGUGGAGGGCAGUGGGGGUACCAUA	30N	110
74A	GGGGUCAAGGACAGUGGGUGGUUGGUGGUUGU	30N	111
16B	UGCUGCGGUGCGCAUGUGUGGAAGACAGAGGGAGGUUAUGAAUCUGACGU	50N	112
31B	ACAGACCGUGUGUGGAAGACAGUGGGAGGUUAACGUAGUGAUGGC	50N	113
38B	GCUGCGGUGCGCAUGUGUGGAAGACAGAGGGAGGUUAUGAAUCUGGCCG	50N	114
39B	GAAAACUACGGUGUGUGGAAGACAGUGGGAGGUUGGCAGUCUGUGUCCGU	50N	115

TABLE VIII. (CONTINUED)

## FAMILY 1A

		CORRESPONDING CLONE	SEQ ID NO:
62B	UCCAUCGUGGAAGACAGUGGGAGGUAGAAUCAUGACGUACAGACGACUC	50N	116
79B	UGUGAUUUGUGUGGAAGGCAGUGGGAGGUGCGAUGUAGAUCUGGCGAUG UGUGUGGAAGACAGUGGGWGGUU	50N	117
		★	118
FAMILY 1B			
59A	UGUGUGGAAGGGUACCUGAGU---GGGGAUAGGG	30N	119
82A	AAGACUGUGUGGAAGGG---UGUA---GGGGUUGGG	30N	120
3B	UAGGGCCGCAACUGUGUGGAAGGGAGGAUGCGCUAUGGGGUUGGGCUG UGUGUGGAAGGGNNNNUGNGU---GGGGUUGGG	50N	121
		★	122
FAMILY 1C			
1B	AUUGUGUGGGAUAG-GGCAUAGA-GGGUGU-GGGAAACCCCAGACCCGGCGU	50N	123
43B	UGUGUGGGACAGCGG-AUC-AGGGGUGU-GGGAGCGCAUAACAUCUACNUGC	50N	124
30B	ANNNNUNUGCAUGUGUGGGACAG-GGUGCAUGUGGGUUGCGGGACCUUGGU UGUGUGGGACAG-GGNAUANANGGUGU-GGGA	50N	125
		★	126
FAMILY 2			
51A	GCAGGAGGAUAGGAUCGGAUGGGUAGGA	30N	127

		CORRESPONDING CLONE	SEQ ID NO:
FAMILY 2			
53A	UGAGGAUCGGAUGGGGAGCAGGCCGAGAA	30N	128
67A	GUGGAUUGGAAGGGGUGCUGGAGGAGACG	30N	129
15B	UAGGAUUGGAUGGGGUUGGAACAGAGUUCUAAUGUCGACCUCACAUUGGG	50N	130
77B	CAGGAUUGGAUGGGGUUGGAACAGAGUUCUAAUGUCGACCUCACAUUGGG	50N	131
48B	CAGGAUAGGAUGGGGUCGGAACCGUGUAUCAUAACGAGUCAUCUCCUGGU GGAUHGGGAUGGGGU	50N	132
		★	133
FAMILY 3			
58A	UUAACGGGGUGGUCCGAGGGUGGGAGAAC	30N	134
64A	GACUAGGGCGGGACCGUGGGUGGUGAGUGG	30N	135
50B	AGUGGCAUGGGCCUGGGAGGUGAGUGUCGAGACUGGUGUUGGGCU	50N	136
22B	CGUGGUUCCGUGGGUGGUGAGAGACUUAUCAGUUCGUAGACCGGU CCGUGGGUGGUGAGU	50N	137
		★	138
TWO-MEMBER FAMILIES			
35B	NAAAUAACGAGAGAGGANCAUANNUGACUGAACAUUGAUGUAUUAACGAGU	50N	139
49B	GAGGUACGAGAGAGGAGCGUAGGUGACUGAACAUUGAUGUAUUAACGAGU	50N	140
47B	AGGGUGGCUGGGAGGACCCGGGGUGAAUCGGUAGCACAGUGAUGUUCGGU	50N	141
73B	AGGGUGGCAGGGAGGACCCGGGGUGAAUCGGUAGCACAGUGAUGUUCGGU	50N	142
6A	CGCGAGGGCUGGGGGGUAGGAUGGGUAGA	30N	143
75B	CGCGAGGCUACGAGGCGUGGGGGUGGAAACUAGUUGUGGUCCUCUGGCC	50N	144

TABLE VIII. (CONTINUED)

## TWO-MEMBER FAMILIES

		CORRESPONDING CLONE	SEQ ID NO:	MO 52183
55A	GAUUGGAACCGCUGGGGUUAGGAGGGC	30N	145	
21B	GACCACAGUUAAAACGCCAACUGGGUACGGGUGGGUAAGGAGGGCUG	50N	146	
<b>OTHER SEQUENCES</b>				
6A	CGCGAGGGCUGGCAGGGUAGGAUGGGUAGA	30N	147	
9A	UGGCCCGCCGGUCUUGGGUGUAUGUGUGAA	30N	148	
52A	AGUUGGGGGCUCGUCCGGCGUGGGCGUGC	30N	149	
62A	GGGAUGGUUGGAGACCGGGAGAUGGGAGGA	30N	150	
69A	AAACGGGGCGAUGGAAAGUGGGGUACGA	30N	151	
73A	GAGGAGGAUGGAGAGGAGCGGUGUGCAGGG	30N	152	-85-
83A	GAGAGGGUGAAGUGGGCAGGAUGGGGUAGG	30N	153	
8B	CUGAAAUUGCGGGUGUGGGAGGUUAUGCUGGGAAAGGUGGAUGGUACACGU	50N	154	
13B	CAAUGUUUJGGAGUCUGCUAAUGUGGGUGGUAGACGUACCGAUGGUUGC	50N	155	
14B	ACGGGGAAGUACGAGAGCGGACUGUAAGCUAUGUGGGUCAGUUCGGUG	50N	156	
19B	UUCAGCGCGCAUUAUGUGCGAGCGGGUUCACAAAAGAGGUGUUCGUGUG	50N	157	
26B	CGGAUUGUGUGGUCCGGGAGGGCAGUAGUUUACACUCACCCGUGGUUCGU	50N	158	
29B	GGUGUGUGACAUGUGCGUGGGUUGGGCAGGUACAAAGCGUAUGGGCGUG	50N	159	
34B	AAACGGGAGGUACGAGAGCGGGAGCGCAUAUAUAGGAAACCUUCUUGCACGU	50N	160	
36B	AGGCAGUAIUUGGGGUGGUACAGCGCCUCCCCAAAACUCGCACCUUAGCCC	50N	161	

TABLE VIII. (CONTINUED)

## OTHER SEQUENCES

		CORRESPONDING CLONE	SEQ ID NO:	MO 52183
44B	GGGUUGGGUGGCAAGCGGAGAGCAGGGUUAGGUGCGGACUCAUUGGUGUG	50N	162	
52B	GGAGGGCAGGUUCGAUGCGGGAGCGACUGACCACAGAGAAAUGUGCGGGU	50N	163	
72B	CUCAGCAUCCAGGAAGGGACUUGGUAGGGCACCAUCGAGAUCUUGGCGU	50N	164	
78B	ACCCUAGGCAUCCAGGUUGGGAUAGCGGUUGGAGUGAAUGGUUUGGCC	50N	165	
<b>NITROCELLULOSE-BINDING FAMILY</b>				
5A	CACGGAGGGAGGUUCAGACUUAGCGGUCA	30N	166	
16A	UACAGGGGAAGGAGNGAAUJGCAAGAUGAA	30N	167	
17A*	AAAGUUGUGUGGAAGACAGUGGGAGGUAA	30N	168	-86-
19A	UGAUGGCGGUAGUGGGAGGUAAUGAGCGUNA	30N	169	
25A	UAGGAGGUUGGGAGGAAGCUUCACAGCGGA	30N	170	
40A	UGAGGAGGGAGGAGCACAGGAUUCACGAGU	30N	171	
65A	GUUAGGAGGGUGGUUCAGUGUGGGCAA	30N	172	
66A	CGUCGAGUGCGAUGGGAGGAGGGAGGUCA	30N	173	
74A*	GGGGUCAAGGACAGUGGGUGGUUGGGUGU	30N	174	
75A	GGAGGGAGGGAGGAUGAGGUCAUCAGC	30N	175	
76A	CAAACAGGGAGGAUGGAGGGNG	30N	176	
77A	AGGGGUGGUCCGUAGCUCGGUGGUUGGU	30N	177	
78A	AGGAGGGUUAAGGAGGGAGAUUAAGCGUUGG	30N	178	

TABLE VIII. (CONTINUED)  
NITROCELLULOSE-BINDING FAMILY

		CORRESPONDING CLONE	SEQ ID NO:
81A	GUGGAGGGUACGUGGAGGGAGAGCGACA	30N	179
85A	AUAAAUCAAGGAGGUGGAGGGACAGAUGC	30N	180
86A	GAUGAGGACUCGGGGCGGGAGGGUGGUACCA	30N	181
5B	AGGUCGUGGCUGGGAUUCGUCCUCGACAUGUACAUUGUGGCCUCUGGUGGCC	50N	182
6B	AAGUUAGUCAUCGUGCAAACUGCGAGUGCACUGCUCGGGAUCC	50N	183
21B	GACCACAGUUAAAACGCCAACAGUGGUAGGGUGGGUAAGGAGGGCTG	50N	184
75B	CGCGAGUGCACGAGGCGUGGGGGGGUGGAAACUAGUUGUGCUCUGGCCG	50N	185

★ CONSENSUS SEQUENCE

\* NUCLEOTIDE ABBREVIATIONS C AND U ACTUALLY DEPICT THE MODIFIED NUCLEOTIDES 2'-NH<sub>2</sub>-C AND 2'-NH<sub>2</sub>-U.WO 9521853  
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TABLE IX. DISSOCIATION CONSTANTS FOR A REPRESENTATIVE SET OF HIGH-AFFINITY 2'-NH<sub>2</sub> RNA LIGANDS TO bFGF.

CLONE	Kd (nM)	SEQ ID. NO:
21A	1.3 ± 0.1	104
49A	1.4 ± 0.3	107
53A	1.5 ± 0.3	128
54A	1.7 ± 0.3	108
58A	1.4 ± 0.3	134
59A	1.2 ± 0.2	119
22B	2.8 ± 0.5	137
34B	2.0 ± 0.4	160
47B	2.9 ± 0.3	141
48B	6.7 ± 1.1	132
52B	2.3 ± 0.3	163
72B	3.4 ± 0.5	164
starting random RNA A	65 ± 11	
starting random RNA B	240 ± 140	

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TABLE X. INHIBITION OF RAT CORNEAL VASCULAR  
INGROWTH BY RNA LIGAND 21A.

Day	Group I (untreated)	Group II 21A	Group III (bFGF)	Group IV (21A + bFGF)
7	367 ± 4	363 ± 3	972 ± 72	623 ± 122*
14	470 ± 57	388 ± 11	1528 ± 167	900 ± 80*

Data are mean ± STD. Err.

\*P&lt; 0.05 compared with Group III. (T-test, 2 Tailed)

HEXAGENE/FIGURES/FIGURE.10-RAT

TABLE XI. OLIGONUCLEOTIDES USED IN SELEX EXPERIMENTS A AND B  
TO SELECT 2'-NH<sub>2</sub> PYRIMIDINE RNA LIGANDS TO bFGF.

## SELEX EXPERIMENT A

	SEQ ID NO.
Starting RNA* 5'-GGGAGACAAGAAUAACGUCAA [-30N-]UUCGACAGGAGGCUCACAAACAGGC-3'	SEQ ID NO:95
PCR Primer 1 5'-TAATACGACTCACTATAAGGGAGACAAGAAUAACGUCAA-3' T7 Promoter	SEQ ID NO:96
PCR Primer 2 5'-CCCTGTTGTAGCCTCCTGTCGAA-3'	SEQ ID NO:97

## SELEX EXPERIMENT B

	SEQ ID NO.
Starting RNA* 5'-GGGAGGACGAUGCGG [-50N-] CAGACGACTCGCCCCGA-3'	SEQ ID NO:98
PCR Primer 1 5'-TAATACGACTCACTATAAGGGAGGACGAUGCGG-3' T7 Promoter	SEQ ID NO:99
PCR Primer 2 5'-TCGGGCGAGTCGTCTG-3'	SEQ ID NO:100

\* In the randomized region; [-30N-] or [-50N-]; each pyrimidine contains an amino (-NH<sub>2</sub>) functionality at the 2'-position.

HEXAGENE/FIGURES/FIGURE.11-RAT

TABLE XII. THROMBIN RNA BINDING SEQUENCES

CLASS I	1	2	3	SEQ ID NO:
#1	AGAUGCCUGUCGAGCAUGCUG	AGGAUCGAAAGUUAGUAGCUCUUGUUGUUC	GUAGCUAAAACAGCUUUGUCGACGGG	192
#6	AGAUGCCUGUCGAGCAUGCUG	AGGAUCGAAAGUUAGUAGCUCUUGUUGUUC	GUAGCUAAAACAGCUUUGUCGACGGG	192
#13	AGAUGCCUGUCGAGCAUGCUG	AGGAUCGAAAGUUAGUAGCUCUUGUUGUUC	GUAGCUAAAACAGCUUUGUCGACGGG	192
#19	AGAUGCCUGUCGAGCAUGCUG	AGGAUCGAAAGUUAGUAGCUCUUGUUGUUC	GUAGCUAAAACAGCUUUGUCGACGGG	192
#23	AGAUGCCUGUCGAGCAUGCUG	AGGAUCGAAAGUUAGUAGCUCUUGUUGUUC	GUAGCUAAAACAGCUUUGUCGACGGG	192
#24	AGAUGCCUGUCGAGCAUGCUG	AGGAUCGAAAGUUAGUAGCUCUUGUUGUUC	GUAGCUAAAACAGCUUUGUCGACGGG	192
#25	AGAUGCCUGUCGAGCAUGCUG	AGGAUCGAAAGUUAGUAGCUCUUGUUGUUC	GUAGCUAAAACAGCUUUGUCGACGGG	192
#30	AGAUGCCUGUCGAGCAUGCUG	AGGAUCGAAAGUUAGUAGCUCUUGUUGUUC	GUAGCUAAAACAGCUUUGUCGACGGG	192
#2	AGAUGCCUGUCGAGCAUGCUG	UACUGGAUCGAAAGGUAGUAGGCCAGUCAC	GUAGCUAAAACAGCUUUGUCGACGGG	193
#5	AGAUGCCUGUCGAGCAUGCUG	AUAUCACCGAACUUCGAAAGGAAGUAGCUCUG	GUAGCUAAAACAGCUUUGUCGACGGG	194
#9	AGAUGCCUGUCGAGCAUGCUG	CCUUUCCCGGGUUCGAAGUCAAGUAGGCCGG	GUAGCUAAAACAGCUUUGUCGACGGG	195
#10	AGAUGCCUGUCGAGCAUGCUG	CACCCGGAAUCGAAAGUUAGUAGCUCUGAU	GUAGCUAAAACAGCUUUGUCGACGGG	196
#15	AGAUGCCUGUCGAGCAUGCUG	UGUACCGGAUCGAAAGCUAGUAGCCAGUAC	GUAGCUAAAACAGCUUUGUCGACGGG	197
#16	AGAUGCCUGUCGAGCAUGCUG	CAUCCGGAAUCGAAAGUUAGUAGGCCAGUG	GUAGCUAAAACAGCUUUGUCGACGGG	198
#18	AGAUGCCUGUCGAGCAUGCUG	AUUUGUUGCCGAUCAAGUAGUAGGCCUA	GUAGCUAAAACAGCUUUGUCGACGGG	199

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TABLE XII. (CONTINUED)

CLASS I (CONT.)	1	2	3	SEQ ID NO:
#21	AGAUGCCUGUCGAGCAUGCUG	UQUACUGGAUCGAAAGUUAGUAGCCAGUCAC	GUAGCUAAAACAGCUUUGUCGACGGG	200
#22	AGAUGCCUGUCGAGCAUGCUG	AUCGAAGUCAAGUAGGAAAGCUGUO	GUAGCUAAAACAGCUUUGUCGACGGG	201
#26	AGAUGCCUGUCGAGCAUGCUG	ACCCUGOOAGUCGGGAUCGAAAGUAGCUCACU	GUAGCUAAAACAGCUUUGUCGACGGG	202
#31	AGAUGCCUGUCGAGCAUGCUG	GGGUGCGGAUCGAAAGUAGUAGGCCGACU	GUAGCUAAAACAGCUUUGUCGACGGG	203
#33	AGAUGCCUGUCGAGCAUGCUG	AUAUCACCGGAUCGAAAGUAGUAGGCCG	GUAGCUAAAACAGCUUUGUCGACGGG	204
#34	AGAUGCCUGUCGAGCAUGCUG	UGUACUGGAUCGAAAGUAGUAGCAGCAC	GUAGCUAAAACAGCUUUGUCGACGGG	205
#37	AGAUGCCUGUCGAGCAUGCUG	AUAUCACCGGAUCGAAAGUAGUAGGCCG	GUAGCUAAAACAGCUUUGUCGACGGG	206

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## CLASS II

#3	AGAUGCCUGUCGAGCAUGCUG	GUGCCGCUCUUGGGCGCGUUCUOGC	GUAGCUAAAACAGCUUUGUCGACGGG	207
#20	AGAUGCCUGUCGAGCAUGCUG	GUGCCGCUCUUGGGCGCGUUCUOGC	GUAGCUAAAACAGCUUUGUCGACGGG	208
#27	AGAUGCCUGUCGAGCAUGCUG	GUGCCGCUCUUGGGCGCGUUCUOGC	GUAGCUAAAACAGCUUUGUCGACGGG	209
#35	AGAUGCCUGUCGAGCAUGCUG	GUGCCGCUCUUGGGCGCGUUCUOGC	GUAGCUAAAACAGCUUUGUCGACGGG	210
#38	AGAUGCCUGUCGAGCAUGCUG	GUGCCGCUCUUGGGCGCGUUCUOGC	GUAGCUAAAACAGCUUUGUCGACGGG	209
#39	AGAUGCCUGUCGAGCAUGCUG	GUGCCGCUCUUGGGCGCGUUCUOGC	GUAGCUAAAACAGCUUUGUCGACGGG	209

\* THE CONSERVED SEQUENCE MOTIFS WITHIN THE JON VARIABLE REGION ARE UNDERLINED.  
NEXAGEN/FOURSTAR/12-EAU

TABLE XIII. LIGANDS USED IN BOUNDARY EXPERIMENTS

CLONE*	RANDOM REGION	SEQ ID NO:
<b>CLASS I</b>		
6	gggagaugccuguc [g[agcaugcug AGGAUCGAAAGUAGUAGGCCUUUUGUGUCU]C guagcuuacagcuuugucgacggg	211
16	gggagaugccugucgacau [gcug C[AU[CCGGAUCGAAAGUAGUAGGCCGAG]GUG guagcuuacagcuuugucgacggg	212
18	gggagaugccugucgacauugcug AUUGU[UGCGGAUCGAAAGUGAGUAGGGCUA] guagcuuacagcuuugucgacggg	213
<b>CLASS II</b>		
27	gggagaugccuguc {g [agcaugcug GUUCGGCUUUUdggGCCGUCCU] GAC guagcuuacagcuuugucgacggg	214

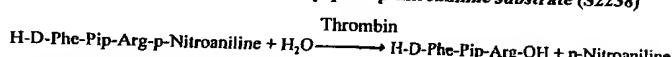
\* NUCLEOTIDES IN THE CONSTANT REGION ARE IN LOWER CASE TYPE.

"I" DENOTES A 5' BOUNDARY AND "J" DENOTES A 3' BOUNDARY

THE PROPOSED 2° STRUCTURES ARE SHOWN IN TABLE XIII.

NEXAGEN/FIGURES/TABLE.13-EAM

TABLE XIV. FUNCTIONAL ASSAYS THROMBIN ACTIVITY

**A. Peptidase Activity-Cleavage of tripeptide p-nitroaniline substrate (S2238)**

	[Thrombin]	[RNA]	Inhibition (decrease in OD <sub>405</sub> )
Class I RNA 16 (SEQ ID NO:198)	10 <sup>-8</sup> M	10 <sup>-8</sup> M	-
	10 <sup>-9</sup> M	10 <sup>-7</sup> M	-
	10 <sup>-9</sup> M	10 <sup>-8</sup> M	-
Class II RNA 27 (SEQ ID NO:209)	10 <sup>-8</sup> M	10 <sup>-8</sup> M	-
	10 <sup>-9</sup> M	10 <sup>-7</sup> M	-
	10 <sup>-9</sup> M	10 <sup>-8</sup> M	-

**B. Fibrinogen Clotting Assay**

Ligand plus purified human thrombin (2.5nM)	Clotting time (sec) for purified fibrinogen (0.25 mg/ml)
--	---

No RNA/DNA	65
Class I RNA 16 (30nM)	117
Class II RNA 27 (60nM)	115
DNA 15mer G15D (SEQ ID NO:189)	270-330

NEXAGEN/FIGURES/TABLE.14-EAM

TABLE XV. HIGH-AFFINITY DNA LIGANDS TO THROMBIN

11TH ROUND 30N SEQUENCES		SEQ ID NO:
5'AGATCCTGTGAGCATGCT (30N)	GTAGCTAAACTCTTGTGACCGG	215
<b>CLONE (30N)</b>		
#1	TCACTAGGGTAGGTGTOCATGATGCTAGTG	216
#2	GTCAGCTACCCGTGTTAGGGAAAGGTTGGAAT	217
#3	ACTAGCGGGTAGTGCTGGTTGGGATCTA	218
#6	ACACCCCTGTTAGGGTAGGATGGGTTGTC	219
#7, 23	GCAGTTGTTGCTGTTGTAAGGTTAGAATGGG	220
#8, 9, 32	GTGAATTAGGTAGGGTCGGATGGCTTACGGT	221
#10	GAATTGAGGGTAGGGCTGGGATGGTGGAAC	222
#13	ATGTGCTACCCGTGTTAGGGAAAGGATGGTGT	223
#14	GTGTTGTTAGGGTAGGGATGGTACGGCTT	224
#15, 34	GTTGGCGGGAGTGGTAGGGCAGTAGGGTTGG	225
#16	GGCCCTACGAGGGTAGGTTGTTGATCTGCC	226
#17	GTTTTGTATTAACCTAGGTGTCATGATCT	227
#18	GTTTATCGGTAGGGTGGTGGCTACAAT	228
#20	ACGGAGCCGCOCAGAACAACTGTGAAAGGCCG	229
#21	ACGTTTGGCTCGGGGTAGGTGGGGTTGGGT	230
#25	GAATCAGTTTGGCTGGTAGGGCAGGTG	231
#26	TAGCTCTCTGTGTTAGGGTAGGGTTGGGATA	232
#27	GCCTAGTGGCGGCCAGCAACTGTGAGGCAC	233
#28	GTGACTACTCTCACCTCTATGGAACGGTCA	243
#29	CGATGGCTGGTAGGGTAGGTGGTGTGTCATT	235
#31	GTTTATCGGTAGGGTGTTGGATGGCTACTTT	236
#33	GCCTTATGGTCCGGGTAGGGTTGGGTTGGA	237
#35	GGAGATGGTAGGGATGGGTTGGACCGCTA	238
#36	GTGAATTAGGTAGGGTCGGATAGGCTACGGT	239

MOMS

TABLE XV. (CONTINUED)

11TH ROUND 60N SEQUENCES		SEQ ID NO:
S'AGATGCCGTGCAAGCATGCT (60N)	GTAGCTAAACTGCTTTTGAGACCGG-3'	240
CLONE (60N)		
#1	GCAAAGCCGGGAAAGTCCCAAGTGGTAGGCTGAGGGTTGGGGATTGTAATCCCTGTGGAC	241
#2	GAAGGCGCAGGGAGGTGGCAACAGGGATGGTTAGTGGCAACATCTGAGATT	242
#3	AGCTTCATCTGCGCCGGTGTGTAAGGGTTGATGCTTGTTAGGCTAGTCCTGAGGCGA	243
#4	CTGGCGGTGGGAGGGAACGGTGTGAGGGCAAGTGGTAGGCTGATGTCACGGCCGTGGCA	244
#5	TGTTGCTGAGCTCTAGATGTAAGGTTATGGGGGTTGGGGTTGGGGATGCGATGCGAG	245
#7	GCGCGCTGGTGTGATGGCGGCACTGGTTGAGGGGAGGAGCTAGATGCTATGAGCG	246
#8	AAAGCCCTGAGGCCGCTGGTTGGGGGGGGTGTGCTAGGTGTGATGATGATCAATACCCACG	247
#9	CCGTGCATCACCGTGCACCGCTGGTTGCTGTTGAGGGAGATGAGCCCAAGACTGG	248
#10	AGCCGAACTTGGCGCTGGTGTACTCGGATTTGGTAAAGCGATGTTGGCTCGGAGTGGCTCGGA	249
#11	TGAGCCAGGTGTTGGGGTTGGGTGCTGCTGAGGGCTCTGATCACCGCGGGGGTGG	250
#12	GGCAGTCCTCTTCGACCAAGGTGTTGGTCTGGCAAGGGAGTGGTTGGATGATGCGGCGA	251
#13	CTAGCCGCTGGTAGGGGGGGTGGGAGTGTGTTGACTCCGCTGGCGTATTCCTGCAAGG	252
#14	CTGGCGCTGGGAGCGGAGGCGCTGATGGCGAACGGTGGAGCTGATGCGGCGGGCGCA	253
#15	GCAGTAGGGAGCAAGCGGGGUCCTAGGGTAAGGTGTTGGATGATCGGGCGGAGGCGCAACTT	254
#16	GGAACTGGGGAGCGCTAGGAGTAAGGATGGGGATGGGGATGGTGGGGGGTTGGCTGTGCA	255

TABLE XV. (CONTINUED)

#	SEQ ID NO:
#18	CTTGGAAAGACGTCGCGTGTAAAGCGCAAGTTGGGTTGACTTCCTGTTGAAAGAAACGGAGACCGT 256
#19	ATGGATAAACACGTCGCCGGAAACGTTGTTAAGGTAGGATGGTGTGATTCGGCCAGGTA 257
#20	CGAGACGGGGGTAGTGTGTTGGATGGGCCCTAGGACATGGCAACTGGCGGTGTTAGCCCTGG 258
#21	GCAAGCCTTCGGTTGTTAATGTTAGGTAGGTTCTTGGTTGGCTGTCTGCTCCACTGTTTC 259
#22	GGCGTCGAGGAACTAATGGTTGTTAGGTGCTTGTTGGCTCTGAAGACGCCGGCTCGTCGCG 260
#24	CCTTGATGGGACGGGGAGGAGTGGGGTGGCGTGTGGCTGACTCOACAGGGTAGCTGGCCACCTC 261
#25	GACGGTGCAGCGGGCGGAACTGGTGTAGGGAGGGTTGGGGCTTCAGCGCTTGTGGGCC 262
#26	CAGCAATGAGGCTCGCGGAGTGTGTTAGGTAGGTTGGGTGTGGAAAGGAGACGAGGGT 263
#27, 32	GGCGTCGCTGATTCGGCTGTGGTAAAGCATTTGGGATGGGCTCTGTGGAGCTCGGCT 264
#28	CGAATGAGGAAACATTGGCGGGCTAAGGTGATTTGTGATGATCGCCGGAGCGGCTGCGACTT 265
#29	GATTGCAATCACTTCGCGGAGTTGTTAAGGGGGTTGGGCGCGGTAGGGCTGGCTAGCC 266
#30	QAGACOTTGGTAAAGGGTGTGTTGGCCCTCGGTGAGGTCCTGCGAAGGCGGGAGTTGTCG 267
#31	GGACACCCCAGGGCTGGCTGGTGTGGAGGCGCTTGGCGATGTGGTAGGGCAAGGACTCGGAT 268
#33	TGTTTCAGTTGTTGGCGGAGGTGGTGTGGATTCAGGATGCGGCAAGGATGTGTGCCCCAC 269
#35	CGGGTATGGCAAGTGGCGCGGCTAGGGCTGTAGGGTGGTGGGCGCCCTGAGCGGCC 270
#36	TGCTGTGCGCTGTTGGACGGGCTGTTAGGGAGGTGGCTGATGTTGGGCGCCCTGAGCGGCC 271

NEXAGENFIGURESTABLE.15-BAS

PLI/US201426

TABLE XVI. STRUCTURE AND DISSOCIATION CONSTANTS ( $K_d$ 's) FOR A REPRESENTATIVE SET OF HIGH-AFFINITY DNA LIGANDS TO THROMBIN

		SEQ ID NO:
30N3	#6	AGATGCCCTGTCGAGCATGCT
	#8	AGATGCCCTGTCGAGCATGCT
	#16	AGATGCCCTGTCGAGCATGCT
	#14	AGATGCCCTGTCGAGCATGCT
	#35	AGATGCCCTGTCGAGCATGCT
60N3	#7	AGATGCCCTGTCGAGCATGCT
	#18	AGATGCCCTGTCGAGCATGCT
	#18(38)	AGATGCCCTGTCGAGCATGCT
	#27	AGATGCCCTGTCGAGCATGCT

Ligand	Kd
30-6	1.2 nM
30-8	0.4 nM
30-14	1.0 nM
30-16	9.4 nM
30-35	1.4 nM
60-7	2.5 nM
60-18	0.92 nM
60-18(38)	1.9 nM
60-27	0.96 nM

### NEXAOENFIGURESTABLE 16-EAM

TABLE XVII. FAMILY 1 RNA LIGANDS TO bFGF.

		SEQ ID NO:
4A	gggagcucagaaaaacgcucaa <u>UGC</u> UA <u>UUCGCC</u> UACU <u>C</u> <u>CGCC</u> <u>CUCC</u> <u>A</u> <u>CC</u> U <u>U</u> ugacaugaggcccggauccggc	281
5A	gggagcucagaaaaacgcucaa <u>AUC</u> <u>CUCC</u> <u>CCC</u> <u>G</u> <u>UCC</u> <u>A</u> <u>GG</u> <u>C</u> <u>U</u> <u>A</u> <u>CC</u> <u>C</u> <u>U</u> <u>G</u> <u>C</u> <u>C</u> <u>A</u> <u>U</u> <u>U</u> ugacaugaggcccggauccggc	282
7A	gggagcucagaaaaacgcucaa <u>UC</u> <u>GG</u> <u>C</u> <u>G</u> <u>A</u> <u>G</u> <u>C</u> <u>U</u> <u>A</u> <u>CC</u> <u>A</u> <u>G</u> <u>A</u> <u>C</u> <u>U</u> <u>C</u> <u>G</u> <u>C</u> <u>U</u> <u>U</u> ugacaugaggcccggauccggc	283
10A	gggagcucagaaaaacgcucaa <u>GU</u> <u>J</u> <u>A</u> <u>G</u> <u>C</u> <u>A</u> <u>C</u> <u>U</u> <u>A</u> <u>U</u> <u>C</u> <u>G</u> <u>C</u> <u>U</u> <u>A</u> <u>CC</u> <u>G</u> <u>G</u> <u>U</u> <u>A</u> <u>G</u> <u>C</u> <u>U</u> <u>C</u> <u>C</u> <u>G</u> <u>A</u> <u>U</u> <u>U</u> ugacaugaggcccggauccggc	284
13A	gggagcucagaaaaacgcucaa <u>A</u> <u>CC</u> <u>CC</u> <u>GG</u> <u>C</u> <u>C</u> <u>U</u> <u>CC</u> <u>G</u> <u>A</u> <u>G</u> <u>C</u> <u>U</u> <u>A</u> <u>AC</u> <u>C</u> <u>AG</u> <u>G</u> <u>A</u> <u>C</u> <u>U</u> <u>U</u> ugacaugaggcccggauccggc	285
14A	gggagcucagaaaa <u>ac</u> <u>cc</u> <u>cu</u> <u>ca</u> <u>a</u> <u>U</u> <u>G</u> <u>G</u> <u>U</u> <u>C</u> <u>A</u> <u>CC</u> <u>A</u> <u>G</u> <u>G</u> <u>A</u> <u>C</u> <u>A</u> <u>CC</u> <u>C</u> <u>A</u> <u>G</u> <u>C</u> <u>U</u> <u>G</u> <u>U</u> <u>U</u> ugacaugaggcccggauccggc	286
16A	gggagcucagaaaaacgcucaa <u>C</u> <u>A</u> <u>C</u> <u>G</u> <u>C</u> <u>A</u> <u>G</u> <u>C</u> <u>U</u> <u>A</u> <u>CC</u> <u>A</u> <u>G</u> <u>C</u> <u>A</u> <u>U</u> <u>G</u> <u>G</u> <u>C</u> <u>C</u> <u>U</u> <u>U</u> ugacaugaggcccggauccggc	287
18A	gggagcucagaaaaacgcucaa <u>U</u> <u>C</u> <u>G</u> <u>G</u> <u>U</u> <u>U</u> <u>G</u> <u>U</u> <u>A</u> <u>U</u> <u>A</u> <u>AC</u> <u>C</u> <u>A</u> <u>G</u> <u>C</u> <u>A</u> <u>U</u> <u>G</u> <u>C</u> <u>C</u> <u>U</u> <u>U</u> ugacaugaggcccggauccggc	288
21A	gggagcucagaaaaacgcucaa <u>U</u> <u>G</u> <u>G</u> <u>U</u> <u>C</u> <u>U</u> <u>A</u> <u>AC</u> <u>C</u> <u>AG</u> <u>G</u> <u>C</u> <u>A</u> <u>CC</u> <u>C</u> <u>A</u> <u>G</u> <u>C</u> <u>U</u> <u>G</u> <u>U</u> <u>U</u> ugacaugaggcccggauccggc	289
25A	gggagcucagaaaaacgcucaa <u>U</u> <u>A</u> <u>G</u> <u>G</u> <u>U</u> <u>U</u> <u>G</u> <u>U</u> <u>A</u> <u>U</u> <u>A</u> <u>AC</u> <u>C</u> <u>A</u> <u>G</u> <u>C</u> <u>A</u> <u>U</u> <u>G</u> <u>C</u> <u>C</u> <u>U</u> <u>U</u> ugacaugaggcccggauccggc	290
29A	gggagcucagaaaaacgcucaa <u>U</u> <u>G</u> <u>C</u> <u>U</u> <u>A</u> <u>U</u> <u>U</u> <u>G</u> <u>U</u> <u>A</u> <u>AC</u> <u>C</u> <u>A</u> <u>G</u> <u>C</u> <u>A</u> <u>U</u> <u>G</u> <u>C</u> <u>C</u> <u>U</u> <u>U</u> ugacaugaggcccggauccggc	291
38A	gggagcucagaaaaacgcucaa <u>A</u> <u>G</u> <u>C</u> <u>U</u> <u>A</u> <u>U</u> <u>U</u> <u>G</u> <u>U</u> <u>A</u> <u>AC</u> <u>C</u> <u>G</u> <u>A</u> <u>CC</u> <u>C</u> <u>U</u> <u>U</u> ugacaugaggcccggauccggc	292
39A	gggagcucagaaaaacgcucaa <u>A</u> <u>CC</u> <u>A</u> <u>C</u> <u>G</u> <u>C</u> <u>U</u> <u>G</u> <u>U</u> <u>A</u> <u>AC</u> <u>C</u> <u>U</u> <u>U</u> ugacaugaggcccggauccggc	293
56A	gggagcucagaaaaacgcucaa <u>C</u> <u>A</u> <u>G</u> <u>C</u> <u>CC</u> <u>C</u> <u>G</u> <u>U</u> <u>C</u> <u>A</u> <u>AG</u> <u>C</u> <u>U</u> <u>A</u> <u>AC</u> <u>C</u> <u>U</u> <u>U</u> ugacaugaggcccggauccggc	294
61A	gggagcucagaaaa <u>ac</u> <u>cc</u> <u>cu</u> <u>ca</u> <u>a</u> <u>U</u> <u>G</u> <u>G</u> <u>U</u> <u>C</u> <u>A</u> <u>CC</u> <u>A</u> <u>C</u> <u>A</u> <u>U</u> <u>C</u> <u>A</u> <u>C</u> <u>G</u> <u>U</u> <u>U</u> ugacaugaggcccggauccggc	295

\* Arrows indicate the double stranded (stem) regions that flank the conserved loop.

Lower case symbols indicate nucleotides in the constant region.

HELA/EM/FIGURES/TABLE\_17-BAM

TABLE XVIII. FAMILY 2 RNA LIGANDS TO bFGF.

		SEQ ID NO:
11A	gggagcucagaaaa <u>ac</u> <u>cc</u> <u>cu</u> <u>ca</u> <u>a</u> <u>G</u> <u>G</u> <u>U</u> <u>A</u> <u>A</u> <u>C</u> <u>G</u> <u>U</u> <u>U</u> - -GACAAGUAC <u>AC</u> <u>C</u> <u>U</u> <u>G</u> <u>C</u> <u>U</u> <u>U</u> ugacaugaggcccggauccggc	296
12A	gggagcucagaaaa <u>ac</u> <u>cc</u> <u>cu</u> <u>ca</u> <u>a</u> <u>G</u> <u>G</u> <u>U</u> <u>A</u> <u>A</u> <u>C</u> <u>G</u> <u>U</u> <u>U</u> - -GACAAGU <u>GC</u> <u>AC</u> <u>CC</u> <u>A</u> <u>C</u> <u>U</u> <u>U</u> ugacaugaggcccggauccggc	297
26A	gggagcucagaaaa <u>ac</u> <u>cc</u> <u>cu</u> <u>ca</u> <u>a</u> <u>G</u> <u>G</u> <u>U</u> <u>A</u> <u>A</u> <u>C</u> <u>G</u> <u>U</u> <u>U</u> - -GCAAGGCAC <u>AC</u> <u>C</u> <u>U</u> <u>U</u> ugacaugaggcccggauccggc	298
27A	gggagcucagaaaa <u>ac</u> <u>cc</u> <u>cu</u> <u>ca</u> <u>a</u> <u>C</u> <u>U</u> <u>C</u> <u>G</u> <u>A</u> <u>G</u> <u>A</u> <u>C</u> <u>G</u> <u>C</u> <u>U</u> <u>U</u> - -GACAAGA-C <u>U</u> <u>U</u> ugacaugaggcccggauccggc	299
47A	gggagcucagaaaa <u>ac</u> <u>cc</u> <u>cu</u> <u>ca</u> <u>a</u> <u>A</u> <u>G</u> <u>U</u> <u>G</u> <u>G</u> <u>A</u> <u>A</u> <u>C</u> <u>G</u> <u>U</u> <u>U</u> - -GACCA <u>CC</u> <u>C</u> <u>U</u> <u>U</u> ugacaugaggcccggauccggc	300
65A	gggagcucagaaaa <u>ac</u> <u>cc</u> <u>cu</u> <u>ca</u> <u>a</u> <u>G</u> <u>G</u> <u>U</u> <u>C</u> <u>A</u> <u>G</u> <u>C</u> <u>U</u> <u>U</u> - -GACAAGUG <u>C</u> <u>AC</u> <u>U</u> <u>U</u> <u>G</u> <u>G</u> <u>U</u> <u>U</u> ugacaugaggcccggauccggc	301
1B	gggaga <u>ug</u> <u>cc</u> <u>u</u> <u>g</u> <u>c</u> <u>u</u> <u>g</u> <u>a</u> <u>c</u> <u>u</u> <u>a</u> <u>C</u> <u>U</u> <u>G</u> <u>U</u> <u>A</u> <u>A</u> <u>C</u> <u>G</u> <u>U</u> <u>U</u> - -GUCAAGUG <u>C</u> <u>A</u> <u>U</u> <u>U</u> <u>g</u> <u>u</u> <u>a</u> <u>c</u> <u>g</u> <u>u</u> <u>u</u> <u>g</u> <u>u</u> <u>c</u> <u>g</u> <u>g</u> <u>g</u>	302
2B	gggaga <u>ug</u> <u>cc</u> <u>u</u> <u>g</u> <u>c</u> <u>u</u> <u>g</u> <u>a</u> <u>c</u> <u>u</u> <u>a</u> <u>G</u> <u>G</u> <u>C</u> <u>A</u> <u>G</u> <u>U</u> <u>U</u> - -GGCGAGACCA <u>U</u> <u>U</u> <u>g</u> <u>u</u> <u>a</u> <u>c</u> <u>g</u> <u>u</u> <u>u</u> <u>g</u> <u>u</u> <u>c</u> <u>g</u> <u>g</u> <u>g</u>	303
6B	gggaga <u>ug</u> <u>cc</u> <u>u</u> <u>g</u> <u>c</u> <u>u</u> <u>g</u> <u>a</u> <u>c</u> <u>u</u> <u>a</u> <u>G</u> <u>G</u> <u>C</u> <u>A</u> <u>G</u> <u>U</u> <u>U</u> - -GACAAGUG <u>C</u> <u>U</u> <u>U</u> <u>g</u> <u>u</u> <u>a</u> <u>c</u> <u>g</u> <u>u</u> <u>u</u> <u>g</u> <u>u</u> <u>c</u> <u>g</u> <u>g</u> <u>g</u>	304
8B	gggaga <u>ug</u> <u>cc</u> <u>u</u> <u>g</u> <u>c</u> <u>u</u> <u>g</u> <u>a</u> <u>c</u> <u>u</u> <u>a</u> <u>A</u> <u>G</u> <u>G</u> <u>G</u> <u>A</u> <u>A</u> <u>C</u> <u>U</u> <u>U</u> - -GUCCGG <u>U</u> <u>A</u> <u>C</u> <u>C</u> <u>T</u> <u>U</u> <u>U</u> <u>g</u> <u>u</u> <u>a</u> <u>c</u> <u>g</u> <u>u</u> <u>u</u> <u>g</u> <u>u</u> <u>c</u> <u>g</u> <u>g</u> <u>g</u>	305
9B	gggaga <u>ug</u> <u>cc</u> <u>u</u> <u>g</u> <u>c</u> <u>u</u> <u>g</u> <u>a</u> <u>c</u> <u>u</u> <u>a</u> <u>AG</u> <u>G</u> <u>G</u> <u>U</u> <u>A</u> <u>A</u> <u>C</u> <u>U</u> <u>U</u> - -GGCAAGC <u>U</u> <u>C</u> <u>A</u> <u>C</u> <u>U</u> <u>U</u> <u>g</u> <u>u</u> <u>a</u> <u>c</u> <u>g</u> <u>u</u> <u>u</u> <u>g</u> <u>u</u> <u>c</u> <u>g</u> <u>g</u> <u>g</u>	306
11B	gggaga <u>ug</u> <u>cc</u> <u>u</u> <u>g</u> <u>c</u> <u>u</u> <u>g</u> <u>a</u> <u>c</u> <u>u</u> <u>a</u> <u>G</u> <u>G</u> <u>G</u> <u>U</u> <u>A</u> <u>A</u> <u>C</u> <u>U</u> <u>U</u> - -GACAAGACCAC <u>U</u> <u>C</u> <u>C</u> <u>A</u> <u>U</u> <u>U</u> <u>g</u> <u>u</u> <u>a</u> <u>c</u> <u>g</u> <u>u</u> <u>u</u> <u>g</u> <u>u</u> <u>c</u> <u>g</u> <u>g</u> <u>g</u>	307
12B	gggaga <u>ug</u> <u>cc</u> <u>u</u> <u>g</u> <u>c</u> <u>u</u> <u>g</u> <u>a</u> <u>c</u> <u>u</u> <u>a</u> <u>AG</u> <u>G</u> <u>G</u> <u>U</u> <u>A</u> <u>A</u> <u>C</u> <u>U</u> <u>U</u> - -GUCAAGUG <u>C</u> <u>A</u> <u>U</u> <u>U</u> <u>g</u> <u>u</u> <u>a</u> <u>c</u> <u>g</u> <u>u</u> <u>u</u> <u>g</u> <u>u</u> <u>c</u> <u>g</u> <u>g</u> <u>g</u>	308

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TABLE XVIII. (CONTINUED)

		SEQ ID NO:
13B	gggagaugccugucgagca <u>uqcu</u> ggAACGCUAUC-GACGAGUGCACCCGGCA <u>guag</u> cuuacagcuuugug <u>ucgac</u> ggg	309
14B	gggagaugccugucgagca <u>uqcu</u> GGGUACGUUGG-GUCAACCA <u>CCUC</u> gu <u>uac</u> aa <u>ac</u> guuu <u>uguc</u> gacggg	310
15B	gggagaugccugucgagca <u>uqcu</u> GGGUACGUAUU-GGCAAGG-CACCG <u>GC</u> gu <u>uac</u> aa <u>ac</u> guuu <u>uguc</u> gacggg	311
19B	gggagaugccugucgagca <u>uqcu</u> GGGUACGUUGG-GACAAGUGCAC <u>CCAGC</u> u <u>uac</u> gu <u>uac</u> guuu <u>uguc</u> gacggg	312
22B	gggagaugccugucgagca <u>uqcu</u> GGGUACGUACU-GGCAAGGU <u>ACCU</u> CAG <u>CC</u> gu <u>uac</u> guuu <u>uguc</u> gacggg	313
28B	gggagaugccugucgagca <u>uqcu</u> GGGUACGUAUU-GUCAGA-CAC <u>CC</u> u <u>uac</u> gu <u>uac</u> guuu <u>uguc</u> gacggg	314
29B	gggagaugccugucgagca <u>uqcu</u> GGGUACGUAUU-GGCAAGA-CAC <u>CC</u> AG <u>CCC</u> gu <u>uac</u> guuu <u>uguc</u> gacggg	315
36B	gggagaugccugucgagca <u>uqcu</u> GGGUACGUACC-GUCGAC-CAC <u>CC</u> u <u>uac</u> gu <u>uac</u> guuu <u>uguc</u> gacggg	316
38B	gggagaugccugucgagca <u>uqcu</u> GGGUACGUUGA-GUCAAGUGCA <u>CCUG</u> AC <u>AC</u> gu <u>uac</u> guuu <u>uguc</u> gacggg	317
48B	gggagaugccugucgagca <u>uqcu</u> GGGUACGUUGU-GACAAGAU <u>ACCC</u> AG <u>UUU</u> gu <u>uac</u> guuu <u>uguc</u> gacggg	318
49B	gggagaugccugucgagca <u>uqcu</u> GGGUACGUUGU-GACAAGUGCAC <u>CCU</u> u <u>uac</u> gu <u>uac</u> guuu <u>uguc</u> gacggg	319

\* Arrows indicate the double stranded (stem) regions that flank the conserved loop.  
Lower Case symbols indicate nucleotides in constant region.

HEXAGEN/FIGURES/TABLE 18-EAM

TABLE XIX. OLIGONUCLEOTIDES USED IN SELEX EXPERIMENTS 1, 2 AND 3 TO SELECT DNA LIGANDS TO bFGF

## EXPERIMENT 1

		SEQ ID NO:
5p2	ATCCGCCCTGATTAGCGATACT	321
40N2	ATCCGCCCTGATTAGCGATACT (40N) ACTTGAGCAAATCACCTGCAGGGG	322
3p2	TGA <u>ACTCGTTTACTGGACGTCCCCJJJ</u>	323

## EXPERIMENT 2

5pBH1	CTACCTACGATCTGACTAGC	324
40NBH1	CTACCTACGATCTGACTAGC (40N) TAGCTTACTCTCATGTATTCC	325
3pBH1	ATCGAATGAGAGTACATAAGGJAJA	326

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## EXPERIMENT 3

5p7.1PS	GGGAGGACGATGCCG	327
30N7.1PS	GGGAGGACGATGCCG (30N) CAGACGACGACGGGG	328
3p7.1PS	GTCTGCTGCTGCCCTJAJA	329

J = BIOTIN

HEXAGEN/FIGURES/TABLE 19-EAM

TABLE XX. AFFINITY OF DNA LIGANDS TO bFGF AFTER EACH ROUND OF SELEX

## Experiment 3 DNA SELEX

Round	% Bound to bFGF	% Bound to Nitrocellulose (Background)	[bFGF] nM	[DNA] nM	Kd nM	
0	10	59	500	1000	~300nM	
1	4.8	14.5	250	1000		-103-
2	5.9	32.5	250	1000		
3	5	8.9	100	500		
4	6	89	100	500		
5	1.1	19.2	33	167		
6	2.1	9.7	50	250		
7	2.8	3.2	33	167		
8	1.7	5.4	20	100	28 nM	
9	2.5	10.8	1	5		
10	1.6	6.9	1	5	2.5 nM	Clone
11	1.1	7	1	5	4 nM	

NEXAGEN/FIGURE/TABLE/XX-EAM

TABLE XXI.

FAMILY 1  
ALIGNED SEQUENCE GROUP: 30 SEQs, 0.52 AVG. IDENTITY  
EXPERIMENT 1 Sequences

		SEQ ID NO:
D3 *	ATCCGGCTGATTAGCGATACTgtgcgatta	330
D10 *	ATCCGGCTGATTAGCGATACTaaggcc	331
D12 *	ATCCGGCTGATTAGCGATACTaaggcc	332
D22	ATCCGGCTGATTAGCGATACTcggc	333
D8	ATCCGGCTGATTAGCGATACTcggc	334
D42	ATCCGGCTGATTAGCGATACTcggc	335
D5	ATCCGGCTGATTAGCGATACTcgcttc	336
D19 *	ATCCGGCTGATTAGCGATACTcgcttc	337
D36	ATCCGGCTGATTAGCGATACTca	338
D43	ATCCGGCTGATTAGCGATACTtagt	339
Consensus	RGGGCTTNGCAAAN	340
Truncation	(D12t2) AGGCC AGGGCTATGCAAAT	341

## EXPERIMENT 2 Sequences

b22	CTACCTACGATCTGACTA	GCagggctttgttaaac atggactacgtacactatgcaggcaaTAGCTTACTCTCATGTAFTTCC	342
b26	CTACCTACGATCTGACTA	GCagggctttgttaaac atggactacgtacactatgcaggcaaTAGCTTACTCTCATGTAFTTCC	343
b28	CTACCTACGATCTGACTA	GCagggctttgttaaac atggactacgtacactatgcaggcaaTAGCTTACTCTCATGTAFTTCC	344
b32	CTACCTACGATCTGACTA	GCagggctttgttaaac atggactacgtacactatgcaggcaaTAGCTTACTCTCATGTAFTTCC	345
b5	CTACCTACGATCTGACTA	GCagggctttgttaaac atggactacgtacactatgcaggcaaTAGCTTACTCTCATGTAFTTCC	346
b7	CTACCTACGATCTGACTA	GCagggctttgttaaac atggactacgtacactatgcaggcaaTAGCTTACTCTCATGTAFTTCC	347
b13	CTACCTACGATCTGACTA	GCagggctttgttaaac atggactacgtacactatgcaggcaaTAGCTTACTCTCATGTAFTTCC	348
b14	CTACCTACGATCTGACTA	GCagggctttgttaaac atggactacgtacactatgcaggcaaTAGCTTACTCTCATGTAFTTCC	349
b15	CTACCTACGATCTGACTA	GCagggctttgttaaac atggactacgtacactatgcaggcaaTAGCTTACTCTCATGTAFTTCC	350
Consensus	GCAGGGCTTNGCAAAN		351

\* Molecules tested for affinity to bFGF

TABLE XXI. (CONTINUED)

FAMILY 1 (CONTINUED)		SEQ ID NO:
EXPERIMENT 3 Sequences		
M17	*	GGGAGGACGATGC GGggggcttgcaaaa atgttaatctacc CAGACGACGACGGGA 352
M19	*	GGGAGGACGATC CGGGggctgtataat tactgtgtactacgtat CAGACGACGACGGGA 353
M23		GGGAGGACGATGCC ggggggctctgtaaag tcttcataatccac CAGACGACGACGGGA 354
M24		CGAGGAGCATGCG CGGgggtctgcataag ttaaaatccccactacccg CAGACGACGACGGGA 355
M210		GGGAGGACGATGC GGggggctgtcaaaaa ttctgttactactcg CAGACGACGACGGGA 356
M217		GGGAGGACGATGCCGGggctacgtc cggggggctttgtaaaaa ccccg CAGACGACGACGGGA 357
M222		GGGAGGACGATGCG CGGGggctgtcaataat ttccaaactatcgat CAGACGACGACGGGA 358
M225	*	GGGAGGACGATGCCGGggctacgtc cggggggctttgtaaaaa ccccg CAGACGACGACGGGA 359
M235	*	GGGAGGACGATCGGGggctacgtc cggggggctttgtaaaaa gacacaggcttcatcgat CAGACGACGACGGGA 360
M236		GGGAGGACGATCGGGggctgtcaaat cctctctcgggaggctacg CAGACGACGACGGGA 361
M242		GGGAGGACGATCGGGggctttgtaaaaa tctctatcgagactacgt CAGACGACGACGGGA 362
Consensus		SSGGGGCTTNGCAAN 363
Truncation	(M225t3)	GCGGGGCTACGTAC CCGGGCTTGTAAAAA CCCCCG 364
Truncation	(m19t2)	G CGGGGCTATGTAAAAT TACTGCTGTACTACGATC 365

\* Molecules tested for affinity to bPGF

TABLE XXI. (CONTINUED)

FAMILY 2

**ALIGNED SEQUENCE GROUP: 24 SEQs; 0.42 AVG. IDENTITY**

## **EXPERIMENT 1 Sequences**

## **EXPERIMENT 2 Sequences**

b19	CTACCTACGATCTGACTAGCttggaggcggtt	cctggacagttttctgagagTAGCTTACTCTCATGTATPTCC	378
b23	CTACCTACGATCTGACTAGCttggaggcggtt	cctggacagttttctgagagTAGCTTACTCTCATGTATPTCC	379
b29	CTACCTACGATCTGACTAGCttggaggcggtt	cctggacagttttctgagagTAGCTTACTCTCATGTATPTCC	380
b33	CTACCTACGATCTGACTAGCttggaggcggtt	cctggacagttttctgagagTAGCTTACTCTCATGTATPTCC	381
b25	CTACCTACGATCTGACTAGCttggaggcggtt	cctggacagttttctgagagTAGCTTACTCTCATGTATPTCC	382
b3	CTACCTACGATCTGACTAGCttggaggcggtt	cctggacagttttctgagagTAGCTTACTCTCATGTATPTCC	383

## **EXPERIMENT 3 Sequences**

m2	GGGAGGACGATCGGCGacatagacgtcgaggaaatcttttagtgcgaCAGACAGCAGCGGGGA	384
m215	GGGAGGACGATCGGCGacagggcaggggccacaatcgatctcgatCAGACAGCAGCGGGGA	385
m228	GGGAGGACGATCGGCGacggggctttagccggccggacaaaatCAGACAGCAGCGGGGA	386
m234	GGGAGGACGATCGGCGacggggctttagccggccggacggatCAGACAGCAGCGGGGA	387
m237	GGGAGGACGATCGGCGacggggctttagccggccggggcaatCAGACAGCAGCGGGGA	388
m250	GGGAGGACGATCGGCGactgtacgatcgatgtacttcgttccAGACAGCAGCGGGGA	389
43 Consensus <sub>88</sub>	CAGAGGAT-CTTTAGCGCCACAGGTT	390
44 Truncation (234:t2)	CAGAGGAT-CTTTAGCGCCACAGGTT	391

\* Molecules tested for affinity to bFGF

TABLE XXI. (CONTINUED)

## FAMILY 3

ALIGNED SEQUENCE GROUP: 18 SEQs, 0.42 AVG. IDENTITY

## EXPERIMENT 1 Sequences

		SEQ ID NO:
d7	ATCCGCTGATTAGCGATACTtgagtgcatcgaccatcgccatgggtatAGCTGAGCAAATCACCTGCAGGGG	392
d13	ATCCGCTGATTAGCGATACTtgccatgggtatAGCTGAGCAAATCACCTGCAGGGG	393
d17	ATCCGCTGATTAGCGATACTacaaggcaaccccgatcacataggcttcgcttaactgcacacgACTTGAGCAAATCACCTGCAGGGG	394
d21	ATCCGCTGATTAGCGATACTtgactgt ggtcacccctggcggaaaacccaggtaaaactcaACTTGAGCAAATCACCTGCAGGGG	395
d25	ATCCGCTGATTAGCGATACTtgactgt ggtcacccctggcggaaaacccaggtaaaactcaACTTGAGCAAATCACCTGCAGGGG	396
d32	ATCCGCTGATTAGCGATACTtgccatggcaagatctccggcgttgtatcccgtatcgACTTGAGCAAATCACCTGCAGGGG	397
d41	ATCCGCTGATTAGCGATACTtgccatggcgttgtataggctgcacatACTTGAGCAAATCACCTGCAGGGG	398

## EXPERIMENT 2 Sequences

b18	CTACCTACGATCTGACTAGCtaccacatgtgcaggctttcgccggccactgggttgtAGCTTACTCTCATGTAPTTCC	399
b31	CTACCTACGATCTGACTAGCtactgtgcgtccatctcgactgaaagtccaggTTAGCTTACTCTCATGTAPTTCC	400
b35	CTACCTACGATCTGACTAGCtactgtgcgtccatctcgactgaaagtccaggTTAGCTTACTCTCATGTAPTTCC	401
b1	CTACCTACGATCTGACTAGCtactgcacacccgttatggaggcTAGCTTACTCTCATGTAPTTCC	402
b16	CTACCTACGATCTGACTAGCactgatcccgagactggccctcgccgtgtgaatcgaccaTAGCTTACTCTCATGTAPTTCC	403

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## EXPERIMENT 3 Sequences

m202	GGGAGGACGATGCCGtccgggtataaggcttagggttcgttacAGACGACGACGGGA	404
m203	GGGAGGACGATGCCGtccgggttttcgttacAGACGACGACGGGA	405
m208	GGGAGGACGATGCCGtccgggttttcgttacAGACGACGACGGGA	406
m219	GGGAGGACGATGCCGtccgggttttcgttacAGACGACGACGGGA	407
m227	GGGAGGACGATGCCGtccgggttttcgttacAGACGACGACGGGA	408
m233	GGGAGGACGATGCCGtccgggttttcgttacAGACGACGACGGGA	409

\* Molecules tested for affinity to bPGF

TABLE XXI. (CONTINUED)

## FAMILY 4

ALIGNED SEQUENCE GROUP: 13 SEQs, 0.47 AVG. IDENTITY

## EXPERIMENT 1 Sequences

		SEQ ID NO:
d33	ATCCGCTGATTAGCGATACTtgagcaactcgccatggcagatcgctatcccAGCTGAGCAAATCACCTGCAGGGG	410
d49	ATCCGCTGATTAGCGATACTtgagcaactcgccatggcagatcgctatcccAGCTGAGCAAATCACCTGCAGGGG	411

## EXPERIMENT 2 Sequences

b17	CTACCTACGATCTGACTAGCaaaggatgtaaacaccttacatcgagggtccggccaaacagTAGCTTACTCTCATGTAPTTCC	412
b20	CTACCTACGATCTGACTAGCtacccgttccggatggatccggatgtAGCTTACTCTCATGTAPTTCC	413
b8	CTACCTACGATCTGACTAGCccatcgataggatccggatgtatgtatgtAGCTTACTCTCATGTAPTTCC	414
b10	CTACCTACGATCTGACTAGCccatcgataggatccggatgtatgtatgtacccAGCTTACTCTCATGTAPTTCC	415

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## EXPERIMENT 3 Sequences

m15	GGGAGGACGATGCCGgaggactcgatccggccatcgacttgcAGACGACGACGGGA	416
m29	GGGAGGACGATGCCGtccggatggatccggatgtAGCTGAGCAAATCACCTGCAGGGG	417
m221	GGGAGGACGATGCCGtccggatggatccggatgtAGCTGAGCAAATCACCTGCAGGGG	418
m48	GGGAGGACGATGCCGtccggatggatccggatgtAGCTGAGCAAATCACCTGCAGGGG	419
m247	GGGAGGACGATGCCGtccggatggatccggatgtAGCTGAGCAAATCACCTGCAGGGG	421
m249	GGGAGGACGATGCCGtccggatggatccggatgtAGCTGAGCAAATCACCTGCAGGGG	422

\* Molecules tested for affinity to bPGF

TABLE XXI. (CONTINUED)

**FAMILY 5**  
**ALIGNED SEQUENCE GROUP: 10 SEQs, 0.42 AVG. IDENTITY**

## **EXPERIMENT 1 Sequence:**

EXPERIMENT	SEQUENCES	SEQ ID NO:
d1 *	ATCCGCTGTATTAGCGATACTacacccaaaccttcaagattttagagcaactcgccgcacACTTGAGCAAAATCACCTGCAGGG	423
d9 *		
ATCCGCTGTATTAGCGATACTacacccaaaccttcaagattttagagcaactcgccgcacACTTGAGCAAAATCACCTGCAGGG	424	
d28	ATCCGCTGTATTAGCGATACTacacccaaaccttcaagattttagagcaactcgccgcacACTTGAGCAAAATCACCTGCAGGG	425

## **EXPERIMENT 2 Sequences**

b34 CTACCTACGATCTGACTAGCcaccgaagggtttggatgagggtaggtcaagggtgcggtatccTAGCTTACTCTCATGTAAFTTC  
b2 CTACCTACGATCTGACTAGCcaccgaagctgttccaaaaggctcatagtacccgttcagtcTAGCTTACTCTCATGTAAFTTC

## EXPERIMENT 3 Sequences

DATA ELEMENT 3 Sequences		
m28	GGGAGGACGATCGGacacggctagtcggaggattcaacttccgc	428
m207	GGGAGGACGATCGGccggccatataatgggttatecccgta	429
m224 *	GGCAGAGACCGATCGGcacccggaaataectgcacgcggatggcg	430
m246	GGGAGGACGATCGGccctcagcgattttctggcgtagtgaggcgc	431

\* Molecules tested for affinity to bFGF

1033/0143

WO 95/21853

PCT/US93/01455

TABLE XXI. (CONTINUED)

**FAMILY 5 (CONTINUED)**

### ORPHAN SEQUENCES: (40)

**EXPERMINET 1 Sequences** SEQ ID NO:  
d20 ATCCGCCCTGATTAGCGTACTA taaggccaaacaacgtgaccggggtagaggggttctatc ACTTGACCAAAATCACCTGCAGGGG 432  
d31 \* ATCCGCCCTGATTAGCGTACTA catatccatcgatccggaaatgtttttcaaggccatcg ACTTGACCAAAATCACCTGCAGGGG 433

## EXPERIMENT 2 Sequences

b4 CTACCTAGATCTGACTAGCagctagtgcacttcgagtaaccgagtggttggaaatacgTAGCTTACTCTCATGTAFTTCC  
 b24 CTACCTAGATCTGACTAGCcccttagatgcagtcactgcaggcatgcacgattaccatgcgTAGCTTACTCTCATGTAFTTCC

## EXPERIMENT 3 Sequences

\* Molecules tested for affinity to bFGF

NEXAGEN\FIGURES\TABLE.2IV-EAN

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SEQUENCE LISTING

## (1) GENERAL INFORMATION:

## (i) APPLICANT:

Gold, Larry  
Janjic, Nebojsa  
Trast, Diane(ii) TITLE OF INVENTION: HIGH AFFINITY LIGANDS OF BASIC  
FIBROBLAST GROWTH FACTOR AND  
THROMBIN

## (iii) NUMBER OF SEQUENCES: 445

## (iv) CORRESPONDENCE ADDRESSEEE:

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(B) STREET:

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(D) STATE: Colorado

(E) COUNTRY: USA

(F) ZIP: 80111

(v) COMPUTER READABLE FORM:  
(A) MEDIUM TYPE: Diskette, 3.5 inch, 1.44 MB storage  
(B) COMPUTER: IBM compatible  
(C) OPERATING SYSTEM: MS-DOS  
(D) SOFTWARE: Wordperfect 5.1

## (vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:

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## (vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: 08/195,005

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(A) APPLICATION NUMBER: 08/219,012

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## (ix) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: 07/973,333

(B) FILING DATE: 11-NOVEMBER-1992

(C) CLASSIFICATION:

TABLE XXII.

## ISOLATES AND TRUNCATES WITH THE HIGHEST AFFINITY FOR BFGF

Ligand	K <sub>d</sub> nM	SEQ ID NO:
M17	6.9	352
M19	0.3	353
m26	1.6	436
m206	1.8	438
m224	1.5	430
M225	0.1	459
m234	0.7	487
M235	0.2	460
D12	0.3	432
D19	0.1	437
D3	0.3	430
D10	0.3	431

Truncations		K <sub>d</sub> nM	SEQ ID NO:
M225T3	GCGGGGCTACGTACCGGGCTTTGTAAGAACCCGC	0.7	364
M19T2	GCGGGGCTATGTAATTACTGCTGACTACGCATC	1	365
M235T2	GCGGGGCTCTGCCAAAGGACACAGGTCCTACGCATCA	1	420
D12T2	AGGCCAGGGCTATGCCAAATCGCGGCCCTATGGCC	1	341
m234T2	CGAGGAGCTTAGGCCACAGGTT	6	391
M225T3GC	GGGGGGCTACGTACCGGGCTTGTAAAACCCGCC	0.2	443

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## (vii) PRIOR APPLICATION DATA:

- (A) APPLICATION NUMBER: 07/1536,428  
 (B) FILING DATE: 11-JUNE-1990  
 (C) CLASSIFICATION:

## (viii) ATTORNEY/AGENT INFORMATION:

- (A) NAME: Barry J. Swanson  
 (B) REGISTRATION NUMBER: 33,215  
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## (ix) TELECOMMUNICATION INFORMATION:

- (A) TELEPHONE: (303) 793-3333  
 (B) TELEFAX: (303) 793-3433

## (2) INFORMATION FOR SEQ ID NO:1:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 77 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:  
 CGGAGCTCG AATTAACCTU CAANNNNNN NNUNNNNNN UNNNNNNN  
 NNNUUGACA UGAGGCCGG AUCCGC

50

77

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 48 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:  
 CCGAACTTA ATACACTCA CTATAGGAG CTCAGATAA AGCTCAA

48

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:  
 CGGAGTCA ACCTCTTTA GCTTAC

24

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 79 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:  
 GGAGGAUGC CGUUCAGCAU GUCCGAGCG  
 GGAGGAUGC CGUUCAGCAU GUCCGAGCG  
 NNUNGUACU AAACGACCU GUCCGAGCG

50

79

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## (2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 50 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:  
 CCCGAGCTT ATATAGCTC ACTATAGGAG GATTCCTGTC GACCGATCTC

50

50

## (2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 25 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:  
 CCCTTCGACA ACCTCTTTA GCTTAC

25

25

## (2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 9 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:  
 CTAACCCAGG

9

9

## (2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 30 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:  
 TACCAUCUUGSC CUCACUUGSC GUCCGACCU

30

30

## (2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 30 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:  
 AUCCUCUCC GUCCGACCU ACCUGCCAC

30

30

## (2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 30 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:  
 GGAGGAUGC CGUUCAGCAU GUCCGAGCG  
 GGAGGAUGC CGUUCAGCAU GUCCGAGCG  
 NNUNGUACU AAACGACCU GUCCGAGCG

50

79

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UAGGGGAGGU ACCAACAGACA CGUCGUGGCC

(2) INFORMATION FOR SEQ ID NO:11:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

(2) INFORMATION FOR SEQ ID NO:12:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

(2) INFORMATION FOR SEQ ID NO:13:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

(2) INFORMATION FOR SEQ ID NO:14:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

(2) INFORMATION FOR SEQ ID NO:15:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

(2) INFORMATION FOR SEQ ID NO:16:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

(2) INFORMATION FOR SEQ ID NO:17:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

(2) INFORMATION FOR SEQ ID NO:17:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

(2) INFORMATION FOR SEQ ID NO:18:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

(2) INFORMATION FOR SEQ ID NO:19:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

(2) INFORMATION FOR SEQ ID NO:20:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

(2) INFORMATION FOR SEQ ID NO:21:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

(2) INFORMATION FOR SEQ ID NO:22:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

(2) INFORMATION FOR SEQ ID NO:23:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

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(B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

UGGUGUCUAA CAACCAACCA CUCAGCGGU

(2) INFORMATION FOR SEQ ID NO:3:

(1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

RRGGHAACTT WNNNGCAAGN NCACYY

26

(2) INFORMATION FOR SEQ ID NO:24:

(1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

GGGUUACGCUU GUGACAGAUCA CACCCGCGUC

30

(2) INFORMATION FOR SEQ ID NO:25:

(1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

GGGCACAGCC UACAGACAG AGUCAGCCGAC

30

(2) INFORMATION FOR SEQ ID NO:26:

(1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

CGUCAGAAGG CAACCUAAGG GCGAGCACAC

30

(2) INFORMATION FOR SEQ ID NO:27:

(1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

CGUCAGAAGG CAACCUAAGG GCGAGCACAC

30

(2) INFORMATION FOR SEQ ID NO:28:

(1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

AGUGGAAAC GCUACUGAC AGAACACAC

30

(2) INFORMATION FOR SEQ ID NO:29:

(1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

GGTAAACGUA AATGACAGAU CGACTTGGGG

30

(2) INFORMATION FOR SEQ ID NO:30:

(1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

CUCUGGUAC GCAATGCUCA GUGGCAGTA

30

(2) INFORMATION FOR SEQ ID NO:31:

(1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

AGGGGAAAC GUUGAGACGG GUACACCCUG

30

(2) INFORMATION FOR SEQ ID NO:32:

(1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

ACGAGCTTCG UAACGCUAU CACAGAGUCA

30

(2) INFORMATION FOR SEQ ID NO:33:

(1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

AAGGCGAAC GUUGAGACGG GUACACCCUG

30

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## (2) INFORMATION FOR SEQ ID NO:34:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

## (2) INFORMATION FOR SEQ ID NO:35:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

GAGGUACGU ACTGGCAAG UCACCUAGC

30

## (2) INFORMATION FOR SEQ ID NO:36:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

30

## (2) INFORMATION FOR SEQ ID NO:36:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

30

## (2) INFORMATION FOR SEQ ID NO:37:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

30

## (2) INFORMATION FOR SEQ ID NO:38:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base Pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

30

## (2) INFORMATION FOR SEQ ID NO:39:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base Pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

30

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## UCCGGGUAC GUAUUGGCA GGCACCCAC

## (2) INFORMATION FOR SEQ ID NO:40:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

GGAUAGCG UGGACRAGUG CACCRAGUGC

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

## (2) INFORMATION FOR SEQ ID NO:41:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

AGGUAACGU ACTGGCAAG UCACCUAGC

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

## (2) INFORMATION FOR SEQ ID NO:42:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

AGGUAACGU AUGUCAGA CACCCUAGU

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

## (2) INFORMATION FOR SEQ ID NO:43:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

GGGUACCGA UGGGAAAGC ACCGAGCCC

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

## (2) INFORMATION FOR SEQ ID NO:44:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base Pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

GAGGAAACGU ACCGGAGAC CACGCCAUGC

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

## (2) INFORMATION FOR SEQ ID NO:45:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base Pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

30

30

30

30

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- (1) TOPOLOGY: linear  
AGGUAAACCU GAGUCAGUG CAUCUCAAU
- (2) INFORMATION FOR SEQ ID NO:45:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 30 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:45: 30
- (2) INFORMATION FOR SEQ ID NO:46:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 30 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:46: 30
- (2) INFORMATION FOR SEQ ID NO:47:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 30 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:47: 30
- (2) INFORMATION FOR SEQ ID NO:48:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 30 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:48: 30
- (2) INFORMATION FOR SEQ ID NO:49:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 30 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:49: 30
- (2) INFORMATION FOR SEQ ID NO:50:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 30 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:50: 30
- (2) INFORMATION FOR SEQ ID NO:51:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 30 base pairs
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:51: 30
- (2) INFORMATION FOR SEQ ID NO:52:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 30 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:52: 30
- (2) INFORMATION FOR SEQ ID NO:53:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 30 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:53: 30
- (2) INFORMATION FOR SEQ ID NO:54:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 29 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:54: 29
- (2) INFORMATION FOR SEQ ID NO:55:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 30 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:55: 30
- (2) INFORMATION FOR SEQ ID NO:56:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 30 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:56: 30
- (2) INFORMATION FOR SEQ ID NO:57:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 30 base pairs
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:57: 30

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(1)

SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

30

(2) INFORMATION FOR SEQ ID NO:59:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

30

(2) INFORMATION FOR SEQ ID NO:59:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

30

(2) INFORMATION FOR SEQ ID NO:60:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

30

(2) INFORMATION FOR SEQ ID NO:61:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

30

(2) INFORMATION FOR SEQ ID NO:62:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

30

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(2) INFORMATION FOR SEQ ID NO:63:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

29

(2) INFORMATION FOR SEQ ID NO:64:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

30

(2) INFORMATION FOR SEQ ID NO:65:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

30

(2) INFORMATION FOR SEQ ID NO:66:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

30

(2) INFORMATION FOR SEQ ID NO:67:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

30

(2) INFORMATION FOR SEQ ID NO:68:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

30

(2) INFORMATION FOR SEQ ID NO:69:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

30

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AGGGGAGGG AACGGGACGG CGCGCCGCC

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 74:  
GGGUAAAGGUT GUGACACAUCU CACCUGGGUC

1

**(1)**  
 SEQUENCE CHARACTERISTICS:  
 LENGTH: 30 base pairs  
 TYPE: nucleic acid  
 STRANDEDNESS: single  
 TOPOLOGY: linear  
**(xi)** SEQUENCE DESCRIPTION: SEQ ID NO:69:  
 GCAUGAGGCC GACAGUAGAU ACCGGAUCA

30

(ii) INFORMATION FOR SEQ ID NO:75:  
 (A) SOURCE: CHARACTERISTICS:  
 (A) LENGTH: 30 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 75:  
 GGCTTAAGGG GTCGACACTA CACCTCGCGC

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(x) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GCGGAGCTT GUGCCGGAA GACCGGGGU

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

30

(2) INFORMATION FOR SEQ ID NO:76:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

(x) SEQUENCE DESCRIPTION: SEQ ID NO:71:  
**GGUAAACGUGU GUURACAGUA CACCUUGUC**

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

30

(2) INFORMATION FOR SEQ ID NO:77:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLogy: linear

(xi) SEQUENCE DESCRIPTION: SBR ID NO:77:

(xii) SEQUENCE DESCRIPTION: SEQ ID NO:72:  
 GGGAACGCU GUUAGCAGUA CACCGUC  
 (1) INFORMATION FOR SEQ ID NO:72:  
 (1) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 30 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: Single  
 (D) TOPOLOGY: linear

30

(2) INFORMATION FOR SEQ ID NO: 78:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 78:

(2) INFORMATION FOR SEQ ID NO:73:  
 (1) SEQUENCE CHARACTERISTICS:  
 LENGTH: 30 base pairs  
 TYPE: nucleic acid  
 STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:73::  
 GGGUAGCU GUGACACA CACCGUCU

30

(12) INFORMATION FOR SEQ ID NO:79:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 79:

(2) INFORMATION FOR SEQ ID NO:74:  
(i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 30 base pairs  
    (B) TYPE: nucleic acid  
    (C) STRANDEDNESS: Single

(2) INFORMATION FOR SEQ ID NO:80:  
(i) SEQUENCE CHARACTERISTICS:  
(a) LENGTH: 30 base pairs

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- (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear
- Assucatusc Gtacccgguc AUGCCUGGCC**
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:
- (2) INFORMATION FOR SEQ ID NO:81:
- (1) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 30 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear
- GACGACUCC AAGCAGGAAA CGUACGGCG**
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:
- (2) INFORMATION FOR SEQ ID NO:82:
- (1) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 30 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear
- GAGGCAACAC UACGAAACAG UACCATCCAC**
- (2) INFORMATION FOR SEQ ID NO:83:
- (1) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 30 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear
- GAGGCAACAC UACGAAACAG UACCATCCAC**
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:
- (2) INFORMATION FOR SEQ ID NO:83:
- (1) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 30 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear
- GAGGCAACAC UACGAAACAG UACCATCCAC**
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:
- (2) INFORMATION FOR SEQ ID NO:84:
- (1) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 30 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear
- GAGGCAACAC UACGAAACAG UACCATCCAC**
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:
- (2) INFORMATION FOR SEQ ID NO:85:
- (1) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 30 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear
- GGGGCAACAC UACGAAACAG UACCATCCAC**
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:
- (2) INFORMATION FOR SEQ ID NO:86:

30

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- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 30 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single
- C CGAGGGGUAA CGUUGGGUCA AGCACACC**
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:
- (2) INFORMATION FOR SEQ ID NO:87:
- (1) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 30 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear
- CGGAAACCGU AUCCAGAGU GCACCGCGA**
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:
- (2) INFORMATION FOR SEQ ID NO:88:
- (1) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 30 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear
- CGGAAACCGU AUCCAGAGU GCACCGCGA**
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:88:
- (2) INFORMATION FOR SEQ ID NO:89:
- (1) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 30 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear
- ACTUCUCACTG CGAUCCAAA UCAUGCCUGG**
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:89:
- (2) INFORMATION FOR SEQ ID NO:90:
- (1) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 30 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear
- GGAUGAAGCG GAACTGAGAU AGCGGAUCC**
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:90:
- (2) INFORMATION FOR SEQ ID NO:91:
- (1) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 30 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear
- GCAUGAANGCG GAACTGAGAU AGCGGAUCC**
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:91:
- (2) INFORMATION FOR SEQ ID NO:92:

30



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- (2) INFORMATION FOR SEQ ID NO:102:
- (1) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 30 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (ix) FEATURE:
    - (D) OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:102:  
UGUGUGGAG AGACAGGGG GUUCAGUGU  
30

(2) INFORMATION FOR SEQ ID NO:103:

- (1) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 30 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ix) FEATURE:
  - (D) OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:103:  
AAAGUGUGGU GGAGACAGU GGGAGGUGA  
30

- (2) INFORMATION FOR SEQ ID NO:104:
- (1) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 30 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (ix) FEATURE:
    - (D) OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:104:  
AUAGUGUGGU GGAGACAGUG GGUGGGUGA  
30

(2) INFORMATION FOR SEQ ID NO:105:

- (1) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 30 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ix) FEATURE:
  - (D) OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:105:  
GUAGACATAU GUUGUGGAGA CGCGGGUG  
30

- (2) INFORMATION FOR SEQ ID NO:106:
- (1) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 30 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (ix) FEATURE:
    - (D) OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:106:  
GGGUGUGGAGA AGACAGGGG UGGCUUAGNC  
30

(2) INFORMATION FOR SEQ ID NO:107:

- (1) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 30 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ix) FEATURE:
  - (D) OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:107:  
AUUGUGUGUG GGAGACAGUG GGUGGGUGA  
30

(2) INFORMATION FOR SEQ ID NO:108:

- (1) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 30 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ix) FEATURE:
  - (D) OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:108:  
ACUGUGUGGU GGAGACAGC GGUGGGUGA  
30

(2) INFORMATION FOR SEQ ID NO:109:

- (1) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 30 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ix) FEATURE:
  - (D) OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:109:  
AUUGUAGGU GUUGGGUGA CGUGGGUGG  
30

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(2) **INFORMATION SOURCE**

- (i) **SEQUENCE CHARACTERISTICS:**
  - (A) LENGTH: 30 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single

s are 2'-NH<sub>2</sub> cytosine.

(ix) FEATURE: (xi) SEQUENCE DESCRIPTION: SEQ ID NO:110:  
 (xii) OTHER INFORMATION: All U's are 2'-NH<sub>2</sub> uracil  
 GAUGUGUGGA GGGCACUGGG GGGUACCAUA 30

(ix) FEATURE: All U's are 2'-NH<sub>2</sub> uracil  
(x) OTHER INFORMATION: SEQ ID NO:11:2:  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:2:  
UCCUCGCGUG CGCAAGUG GAAAGCAGAG GGAGGUAGA AUCAUGACCU 50

(ix) LENGTH: 50 base pairs  
 (A) LENGTH: 50 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ix) FEATURE:  
 (D) OTHER INFORMATION: All C's are 2'- $\text{-NH}_2$  cytosine

(ix) FEATURE:  
 (D) OTHER INFORMATION: All U's are 2'- $\text{-NH}_2$  uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:113: 50  
 AGAGACGCG UGGAGAAC AGUUGGAGGU UAUUAUCUA GUGAUGCGC

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(1) SUBSEQUENCE: CTCATGCGATG  
A) LENGTH: 49 base pairs  
B) TYPE: nucleic acid  
C) STRANDEDNESS: single  
D) TOPOLOGY: linear

(1) SUBSEQUENCE CHARACTERISTICS:

- (A) LENGTH: 49 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: All C's are  $2'-\text{NH}_2$ , cytosine

(1) SEQUENCE CHARACTERISTICS:

- LENGTH: 49 base pairs
- TYPE: nucleic acid
- STRANDEDNESS: single
- TOPOLOGY: linear

(ix) FEATURE:

- OTHER INFORMATION: All C's are 2'-NH<sub>2</sub>, cytosine
- FEATURE:
- OTHER INFORMATION: All U's are 2'-NH<sub>2</sub>, uracil
- SEQUENCE DESCRIPTION: SEQ ID NO:14:  
GCTGCGGCG GCAUGUUGAG AAGACGAGG GAGGGUAGAA UCCUGCGCG

(2) INFORMATION FOR SEQ ID NO:15:  
NAME: tRNA<sub>Leu</sub> Lys

(ix) FEATURE: (xi) OTHER INFORMATION: All U's are 2'-NH, uracil  
 (xii) STABILITY DESCRIPTION: SEQ ID NO:115: GAAGACTTACG GTCUGGGAA GACAGCGGA GAGUUGCU CUGUGCCGU 50  
 (xiii) TRANSMISSION FOR SEQ ID NO:116:

(2) INFORMATION FOR SEQ ID NO:117:  
 (3) SEQUENCE CHARACTERISTICS:  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:116:  
 UCCAUCAUGG AAGACAGUG GAGGUUAGAA UCAGAAGCUC AGAACGAC  
 (4) OTHER INFORMATION: All U's are 2'-NH<sub>2</sub> uracil

(C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ix) FEATURE:  
 (D) OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine

(x) FEATURE:  
 (D) OTHER INFORMATION: All U's are 2'-NH<sub>2</sub> uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:  
 UGUGAUUUGU GUUGGAAGCA GUUGGAGGGU UGUAUGURGA UCUGGGCAUG

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- (2) INFORMATION FOR SEQ ID NO:118:
- (1) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 23 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear

(ix) FEATURE:  
 (D) OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:118:  
**UAGUGGAGG ACAGUGGGG GGU**

- (2) INFORMATION FOR SEQ ID NO:119:
- (1) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 30 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear

(ix) FEATURE:  
 (D) OTHER INFORMATION: All U's are 2'-NH<sub>2</sub> uracil  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:119:  
**UGUGUGGAGG GGUACUGGAG UGGUGGGG**

- (2) INFORMATION FOR SEQ ID NO:120:
- (1) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 31 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear

(ix) FEATURE:  
 (D) OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:120:  
**UGUGUGGAGG GGUACUGGAG UGGUGGGG**

- (2) INFORMATION FOR SEQ ID NO:121:
- (1) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 49 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear

(ix) FEATURE:  
 (D) OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:121:  
**AAAGCTTGGGUU GGAGGGGG UGGGGGG G**

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- (2) INFORMATION FOR SEQ ID NO:122:
- (1) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 30 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear

(ix) FEATURE:  
 (D) OTHER INFORMATION: All U's are 2'-NH<sub>2</sub> uracil  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:122:  
**UGUGUGGAGG AGGGAGUCG GGGUGGGG GACGGGGCGU**

- (2) INFORMATION FOR SEQ ID NO:123:
- (1) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 50 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear

(ix) FEATURE:  
 (D) OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:123:  
**AUGUGUGGGG ATAGAGGCA GAGGGUGGG GAMACCCCG AGCGGGCGU**

30

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- (2) INFORMATION FOR SEQ ID NO:124:
- (1) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 51 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear

(ix) FEATURE:  
 (D) OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:124:  
**UGUGUGGAGC AGGGAGUCG GGGUGGGG GACGGGGCGU UCCUAUCUGC**

50

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- (2) INFORMATION FOR SEQ ID NO:125:
- (1) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 50 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear

(ix) FEATURE:  
 (D) OTHER INFORMATION: All U's are 2'-NH<sub>2</sub> uracil  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:125:  
**ANNNNNNUGC AUGUGUGGA CAGGGGGAU GUGGGUGCG GACCGTUGC**

50

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## (2) INFORMATION FOR SEQ ID NO:126:

- (1) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 31 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

## (ix) FEATURE:

- (d) OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine

(x) FEATURE: All U's are 2'-NH<sub>2</sub> uracil

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:126:

UGUGUGGAC AGGGAAUAAA UGGGGUGGG A

## (2) INFORMATION FOR SEQ ID NO:127:

- (1) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 30 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

## (ix) FEATURE:

- (d) OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine

(x) FEATURE: All U's are 2'-NH<sub>2</sub> uracil

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:127:

GCAGGAGGAGT AGGAUUCGCA UGGGGUAGCA

## (2) INFORMATION FOR SEQ ID NO:128:

- (1) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 30 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

## (ix) FEATURE:

- (d) OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine

(x) FEATURE: All U's are 2'-NH<sub>2</sub> uracil

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:128:

UGAGGAUUCG AUGGGAGGACA GGCGGAGGA

## (2) INFORMATION FOR SEQ ID NO:129:

- (1) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 30 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

## (ix) FEATURE:

- (d) OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine

(x) FEATURE: All U's are 2'-NH<sub>2</sub> uracil

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:129:

GUGGAUUGGA AGGGAGGUG GAGGAGGACG

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## (2) INFORMATION FOR SEQ ID NO:130:

- (1) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 50 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

## (ix) FEATURE:

- (d) OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine

(x) FEATURE: All U's are 2'-NH<sub>2</sub> uracil

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:130:

UAGGAUUGGA UGGGGAUUGGA ACAGAGUCU AUAGUCGACC UCAUAGUGG

## (2) INFORMATION FOR SEQ ID NO:131:

- (1) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 50 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

## (ix) FEATURE:

- (d) OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine

(x) FEATURE: All U's are 2'-NH<sub>2</sub> uracil

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:131:

CAGGAUAUGGA UGGGGAUUGGA ACAGAGUCU AUAGUCGACC UCAUAGUGG

## (2) INFORMATION FOR SEQ ID NO:132:

- (1) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 50 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

## (ix) FEATURE:

- (d) OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine

(x) FEATURE: All U's are 2'-NH<sub>2</sub> uracil

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:132:

CAGGAUAUGGA UGGGGAUUGGA ACAGAGUCU AUAGUCGACC UCAUAGUGG

## (2) INFORMATION FOR SEQ ID NO:133:

- (1) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 14 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

## (ix) FEATURE:

- (d) OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine

(x) FEATURE: All U's are 2'-NH<sub>2</sub> uracil

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:133:

GGAUAGAUGU GGGU

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- (2) INFORMATION FOR SEQ ID NO:134:
- (1) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 30 base Pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (ix) FEATURE: OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:134: UURACGGCGU GGUCGAGGCG UGGCGAGUAC

- (2) INFORMATION FOR SEQ ID NO:135:
- (1) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 30 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (ix) FEATURE: OTHER INFORMATION: All U's are 2'-NH<sub>2</sub> uracil
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:134: 30 CCUGGGUGG UGAGU

- (2) INFORMATION FOR SEQ ID NO:136:
- (1) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 47 base Pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (ix) FEATURE: OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:136: GACTUAGCGC GAGCCUGGCG UGGUGAGUG

- (2) INFORMATION FOR SEQ ID NO:137:
- (1) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 47 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (ix) FEATURE: OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:137: 30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:137: UAAUACGG AGAGCAGCU ANNUGACGA ACAGUAGAU AUUAACGAGU

- (2) INFORMATION FOR SEQ ID NO:138:
- (1) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 50 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (ix) FEATURE: OTHER INFORMATION: All U's are 2'-NH<sub>2</sub> uracil
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:138: 15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:139: NAUAUACGG AGAGCAGCU ANNUGACGA ACAGUAGAU AUUAACGAGU

- (2) INFORMATION FOR SEQ ID NO:140:
- (1) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 51 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (ix) FEATURE: OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:140: (xi) SEQUENCE DESCRIPTION: SEQ ID NO:141: 50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:141: GAGGUACGAG AGAGCAGCU AGGGUGACGA ACAGUAGAU AUUAACGUGU

- (2) INFORMATION FOR SEQ ID NO:141:
- (1) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 50 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (ix) FEATURE: OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:141: 50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:142: GAGGUACGAG AGAGCAGCU AGGGUGACGA ACAGUAGAU AUUAACGUGU

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- (2) INFORMATION FOR SEQ ID NO:138:
- (1) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 15 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (ix) FEATURE: OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:138: 15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:139: All U's are 2'-NH<sub>2</sub> uracil

- (2) INFORMATION FOR SEQ ID NO:139:
- (1) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 50 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (ix) FEATURE: OTHER INFORMATION: All U's are 2'-NH<sub>2</sub> uracil
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:139: 15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:140: All U's are 2'-NH<sub>2</sub> uracil

- (2) INFORMATION FOR SEQ ID NO:140:
- (1) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 51 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (ix) FEATURE: OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:140: 50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:141: All U's are 2'-NH<sub>2</sub> uracil

- (2) INFORMATION FOR SEQ ID NO:141:
- (1) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 50 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (ix) FEATURE: OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:141: 50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:142: All U's are 2'-NH<sub>2</sub> uracil

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:137: CGUGGGUGG UGAGU 49
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:138: CGUGGGUGG UGAGU 47
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:139: CGUGGGUGG UGAGU 50
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:140: CGUGGGUGG UGAGU 51
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:141: CGUGGGUGG UGAGU 50
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:142: CGUGGGUGG UGAGU 50

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## (2) INFORMATION FOR SEQ ID NO:142:

## (1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 50 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ix) FEATURE:

## (x) FEATURE:

- (D) OTHER INFORMATION: All U's are 2'-NH<sub>2</sub> uracil

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:142: GAGGGUGGCA GGAGGACCC GCGGUGAUAC GGGUAGCACAG UGAGUUCGU

50

## (2) INFORMATION FOR SEQ ID NO:143:

## (1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ix) FEATURE:

## (x) FEATURE:

- (D) OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine

30

## (2) INFORMATION FOR SEQ ID NO:144:

## (1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 50 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ix) FEATURE:

## (x) FEATURE:

- (D) OTHER INFORMATION: All U's are 2'-NH<sub>2</sub> uracil

30

## (2) INFORMATION FOR SEQ ID NO:145:

## (1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ix) FEATURE:

## (x) FEATURE:

- (D) OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:145: GCGGAGGUGCU AGCAGGCG GGGGGGUGGA AACUAGUUGU GCUUUGGCCG

50

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## (2) INFORMATION FOR SEQ ID NO:146:

## (1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 50 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ix) FEATURE:

## (x) FEATURE:

- (D) OTHER INFORMATION: All U's are 2'-NH<sub>2</sub> uracil

50

## (2) INFORMATION FOR SEQ ID NO:147:

## (1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ix) FEATURE:

## (x) FEATURE:

- (D) OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine

30

## (2) INFORMATION FOR SEQ ID NO:148:

## (1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ix) FEATURE:

## (x) FEATURE:

- (D) OTHER INFORMATION: All U's are 2'-NH<sub>2</sub> uracil

30

## (2) INFORMATION FOR SEQ ID NO:149:

## (1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ix) FEATURE:

## (x) FEATURE:

- (D) OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:149: AQUUUGGGGAGC UCGUGGGGGC UGGGGCGUC

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## (2) INFORMATION FOR SEQ ID NO:150:

## (1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

## (ix) FEATURE:

(D) OTHER INFORMATION: All U's are 2'-NH<sub>2</sub> cytosine

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:150:

GGGAUGGUG GAGACCGGA GAUGGGAGGA

30

## (2) INFORMATION FOR SEQ ID NO:151:

## (1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

## (ix) FEATURE:

(D) OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> uracil

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:151:

AACCGGGCG AUGGAAAGG UGGGGUACCA

30

## (2) INFORMATION FOR SEQ ID NO:152:

## (1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

## (ix) FEATURE:

(D) OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:152:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:152:

30

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## (2) INFORMATION FOR SEQ ID NO:154:

## (1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 49 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

## (ix) FEATURE:

(D) OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:154:

CUGAAAUUGC GGGUGGAGG GUAGUGGG ARAGGUGAU GGUACACGU

49

## (2) INFORMATION FOR SEQ ID NO:155:

## (1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 50 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

## (ix) FEATURE:

(D) OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:155:

CAUUGUUGG AGUCUCUCA UGGGGUGGG UDAGACGAC CGAUGGUAC

50

## (2) INFORMATION FOR SEQ ID NO:156:

## (1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 48 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

## (ix) FEATURE:

(D) OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:156:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:156:

48

## (2) INFORMATION FOR SEQ ID NO:157:

## (1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 50 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

## (ix) FEATURE:

(D) OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:157:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:157:

50

## (2) INFORMATION FOR SEQ ID NO:153:

## (1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

## (ix) FEATURE:

(D) OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine

## (xi) FEATURE:

(D) OTHER INFORMATION: All U's are 2'-NH<sub>2</sub> uracil

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:153:

GAGAGGGUGA AGUGGUGG AGUGGGAGG AUGGGGAGG

30

## (2) INFORMATION FOR SEQ ID NO:151:

## (1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

## (ix) FEATURE:

(D) OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine

## (xi) FEATURE:

(D) OTHER INFORMATION: All U's are 2'-NH<sub>2</sub> uracil

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:151:

TTCAGGGGGC AUUAGUGGAG CGGGGUAC ACAGAGGGUG UUCGGUGUG

50

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## (2) INFORMATION FOR SEQ ID NO:158:

## (1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 50 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ix) FEATURE:

- (x) FEATURE: OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:158: GCGAUGUGU GGUCCGGAGG GCAGUGKUTU ACCACUACACC GUGGUCUGU 50

## (2) INFORMATION FOR SEQ ID NO:159:

## (1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 50 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ix) FEATURE:

- (x) FEATURE: OTHER INFORMATION: All U's are 2'-NH<sub>2</sub> uracil

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:159: GGGUGUGGGG GCAAGCAG AGCAGGUTU GGUGCGSACTU CAUUGUGUG 50

## (2) INFORMATION FOR SEQ ID NO:160:

## (1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 50 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ix) FEATURE:

- (x) FEATURE: OTHER INFORMATION: All U's are 2'-NH<sub>2</sub> cytosine

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:160: GGUGUGUGAC AUAGUGCGC GGUUGGCGCAG GUACRAAGCG UAUGGGCGUG 50

## (2) INFORMATION FOR SEQ ID NO:161:

## (1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 50 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ix) FEATURE:

- (x) FEATURE: OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:161: AACCGGAGGU AGCAGAGGGG GAGGCCATA AUGGGAACTU CTUUGCACU 50

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## (2) INFORMATION FOR SEQ ID NO:162:

## (1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 50 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ix) FEATURE:

- (x) FEATURE: OTHER INFORMATION: All U's are 2'-NH<sub>2</sub> uracil

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:162: GGAGGGGCGAG GUUCCGAUUG GGGAGCAGCAG ACCACAGAGA AUGUGGGGU 50

## (2) INFORMATION FOR SEQ ID NO:163:

## (1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 50 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ix) FEATURE:

- (x) FEATURE: OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:163: CGUAGGAUCC AGGAAGGGA CGUUGGAGGG GACCAUCAG AUGUUGacGU 50

## (2) INFORMATION FOR SEQ ID NO:164:

## (1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 50 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ix) FEATURE:

- (x) FEATURE: OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:164: CGUAGGAUCC AGGAAGGGA CGUUGGAGGG GACCAUCAG AUGUUGacGU 50

## (2) INFORMATION FOR SEQ ID NO:165:

## (1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 50 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ix) FEATURE:

- (x) FEATURE: OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:165: ACCCUAGGUU GGGGGGGUC AGCCCOCCC CAACAUCCG ACCUUAGGCC 50

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- (2) INFORMATION FOR SEQ ID NO:166:

(1) SEQUENCE CHARACTERISTICS:

  - (A) LENGTH: 30 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ix) FEATURE:

  - (D) OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine

(ix) FEATURE:

  - (D) OTHER INFORMATION: All U's are 2'-NH<sub>2</sub> uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:166:  
CAAGCAGGAG GAGGUCAAC UUAGGGGCA 30

(2) INFORMATION FOR SEQ ID NO:167:

(1) SEQUENCE CHARACTERISTICS:

  - (A) LENGTH: 30 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ix) FEATURE:

  - (D) OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine

(ix) FEATURE:

  - (D) OTHER INFORMATION: All U's are 2'-NH<sub>2</sub> uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:167:  
UAGGAGGUG GAGGAAGAUU UCAAGCCCA 30

(2) INFORMATION FOR SEQ ID NO:168:

(1) SEQUENCE CHARACTERISTICS:

  - (A) LENGTH: 30 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ix) FEATURE:

  - (D) OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine

(ix) FEATURE:

  - (D) OTHER INFORMATION: All U's are 2'-NH<sub>2</sub> uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:168:  
UAGGGGAA GGAGGAAUU GCAGAGAA 30

(2) INFORMATION FOR SEQ ID NO:169:

(1) SEQUENCE CHARACTERISTICS:

  - (A) LENGTH: 30 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ix) FEATURE:

  - (D) OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine

(ix) FEATURE:

  - (D) OTHER INFORMATION: All U's are 2'-NH<sub>2</sub> uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:169:  
AAACGUGGUU GGAGAACGUU GGAGGUGAA 30

(2) INFORMATION FOR SEQ ID NO:170:

(1) SEQUENCE CHARACTERISTICS:

  - (A) LENGTH: 30 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ix) FEATURE:

  - (D) OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine

(ix) FEATURE:

  - (D) OTHER INFORMATION: All U's are 2'-NH<sub>2</sub> uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:170:  
CGUAGGAGGG UGGAGGUGG AGUCUGGCA 30

(2) INFORMATION FOR SEQ ID NO:171:

(1) SEQUENCE CHARACTERISTICS:

  - (A) LENGTH: 30 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ix) FEATURE:

  - (D) OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine

(ix) FEATURE:

  - (D) OTHER INFORMATION: All U's are 2'-NH<sub>2</sub> uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:171:  
UGAGGAGGUG GAGGAAGAUU UCAAGCCCA 30

(2) INFORMATION FOR SEQ ID NO:172:

(1) SEQUENCE CHARACTERISTICS:

  - (A) LENGTH: 30 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ix) FEATURE:

  - (D) OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine

(ix) FEATURE:

  - (D) OTHER INFORMATION: All U's are 2'-NH<sub>2</sub> uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:172:  
GUUAGGAGGG UGGAGGUGG AGUCUGGCA 30

(2) INFORMATION FOR SEQ ID NO:173:

(1) SEQUENCE CHARACTERISTICS:

  - (A) LENGTH: 30 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ix) FEATURE:

  - (D) OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine

(ix) FEATURE:

  - (D) OTHER INFORMATION: All U's are 2'-NH<sub>2</sub> uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:173:  
CGUAGGAGGG UGGAGGUGG AGUCUGGCA 30

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- (2) INFORMATION FOR SEQ ID NO:174:
- (1) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 30 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (ix) FEATURE:
    - (x) FEATURE:
      - (D) OTHER INFORMATION: All U's are 2'-NH<sub>2</sub> uracil
    - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:174: GGGUCGAGG ACAGUGGGUG GUGGGUGGU 30
- (2) INFORMATION FOR SEQ ID NO:175:
- (1) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 30 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (ix) FEATURE:
    - (x) FEATURE:
      - (D) OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine
    - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:175: AGGAGGGUUA AGGAGGGACA UTAAACGUG G 30
- (2) INFORMATION FOR SEQ ID NO:176:
- (1) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 23 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (ix) FEATURE:
    - (x) FEATURE:
      - (D) OTHER INFORMATION: All U's are 2'-NH<sub>2</sub> uracil
    - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:176: GGAGGGAGGA GCGAUAAGCA GCUAGCA 30

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- (2) INFORMATION FOR SEQ ID NO:178:
- (1) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 31 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (ix) FEATURE:
    - (x) FEATURE:
      - (D) OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine
    - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:178: AGGAGGGUUA CGUGGAGGG AGAGGCA 31
- (2) INFORMATION FOR SEQ ID NO:179:
- (1) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 29 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (ix) FEATURE:
    - (x) FEATURE:
      - (D) OTHER INFORMATION: All U's are 2'-NH<sub>2</sub> uracil
    - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:179: GUGGAGGGUUA CGUGGAGGG AGAGGCA 29
- (2) INFORMATION FOR SEQ ID NO:180:
- (1) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 30 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (ix) FEATURE:
    - (x) FEATURE:
      - (D) OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine
    - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:180: AUAUATCGAG GAGGUGAGG ACAGAUCC 30
- (2) INFORMATION FOR SEQ ID NO:181:
- (1) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 30 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (ix) FEATURE:
    - (x) FEATURE:
      - (D) OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine
    - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:181: GAUGAGGACTU CGGGGCCAGG GGUGGUACCA 30

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- (2) INFORMATION FOR SEQ ID NO:182:
- (1) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 50 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (ix) FEATURE: OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:182:  
AGGUCUGUCC UGGGAUUCGU CCUGGACAU GUAUAGUUGGG CUCUGUGGCC 50
- (2) INFORMATION FOR SEQ ID NO:183:
- (1) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 43 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (ix) FEATURE: OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> uracil
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:183:  
AGTGUAGUCA UCGUGCAAC UGGGAGUAGCA CUCGUGGGCA UCC 43
- (2) INFORMATION FOR SEQ ID NO:184:
- (1) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 50 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (ix) FEATURE: OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:184:  
AGTGUAGUCA UCGUGCAAC UGGGAGUAGCA CUCGUGGGCA UCC 43
- (2) INFORMATION FOR SEQ ID NO:185:
- (1) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 50 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (ix) FEATURE: OTHER INFORMATION: All U's are 2'-NH<sub>2</sub> uracil
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:185:  
GGCGAGGUGU ACAGGGCGGUG GGGGGUGGGA AACUAGUUGU GCUCUGGGCG 50

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- (2) INFORMATION FOR SEQ ID NO:186:
- (1) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 26 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: Single
    - (D) TOPOLOGY: linear
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:186:  
GGUGUGUGGA AGACAGGGG UGUUUC 26
- (2) INFORMATION FOR SEQ ID NO:187:
- (1) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 27 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: Single
    - (D) TOPOLOGY: linear
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:187:  
GGACGGGGUG GUCCGAGGU GGCGAGGU 27
- (2) INFORMATION FOR SEQ ID NO:188:
- (1) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 38 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: Single
    - (D) TOPOLOGY: linear
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:188:  
GGAGGGACCAU GGCGAACGGG AGGUGAGA GGGGGAGC 38
- (2) INFORMATION FOR SEQ ID NO:189:
- (1) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 15 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:189:  
GGTTGGGTG GTTG 15
- (2) INFORMATION FOR SEQ ID NO:190:
- (1) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 18 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:190:  
GGAUUCGAGNN NAGUAGGC 18
- (2) INFORMATION FOR SEQ ID NO:191:
- (1) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 21 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:191:  
GGCGAGGUGU ACAGGGCGGUG GGGGGUGGGA AACUAGUUGU GCUCUGGGCG 50

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- (2) INFORMATION FOR SEQ ID NO:192:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 75 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear  
     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:192:  
 AGAUGCCGG CGAGCAUCU GAGGAUGAA GUURAGUGC UUUGUGCUU  
 CGUAGCURA CAGGUUGUC GACGG 76
- (2) INFORMATION FOR SEQ ID NO:193:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 74 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear  
     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:193:  
 AGAUGCCGG CGAGCAUCU GUAGCAUGAU GAAGGUNGUA GCGAGUACCG  
 UNGCURAACG AGCUGUGGA CGGG 74
- (2) INFORMATION FOR SEQ ID NO:194:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 75 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear  
     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:194:  
 AGAUGCCGG CGAGCAUCU GAGUAGGAA GUUGGCCUGG  
 GUUGCURAACG ACCUGUCA ACGGG 75
- (2) INFORMATION FOR SEQ ID NO:195:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 76 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear  
     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:195:  
 AGAUGCCGG CGAGCAUCU GUCCUCCG GAGUAGGACU  
 GUUGCURAACG AGCUGUGGC GACGG 76
- (2) INFORMATION FOR SEQ ID NO:196:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 75 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear  
     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:196:  
 AGAUGCCGG CGAGCAUCU GAGCUGAUU AGCGUGAGGU  
 GUACGUAC AGCUGUGGC ACGG 75
- (2) INFORMATION FOR SEQ ID NO:197:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 76 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear  
     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:197:  
 AGAUGCCGG CGAGCAUCU GUAGCAUGAU CGAGUACGU AGGAGGUA  
 CGUAGCURA CAGGUUGUC GACGG 76
- (2) INFORMATION FOR SEQ ID NO:198:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 76 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear  
     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:198:  
 AGAUGCCGG CGAGCAUCU GAGCUGAU CGAGUACGU AGGAGGAGU  
 GUUGCURAACG ACCUGUUC GACGG 76
- (2) INFORMATION FOR SEQ ID NO:199:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 76 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear  
     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:199:  
 AGAUGCCGG CGAGCAUCU GAGUAGGACU  
 GUUGCURAACG AGCUGUGGC GACGG 76
- (2) INFORMATION FOR SEQ ID NO:200:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 76 base pair B  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear  
     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:200:  
 AGAUGCCGG CGAGCAUCU GAGUAGGACU UCGAGAGGAG UAGGAGUA  
 CGUAGCURA CAGGUUGUC GACGG 76
- (2) INFORMATION FOR SEQ ID NO:201:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 65 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear  
     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:201:  
 AGAUGCCGG CGAGCAUCU GAGUAGGACU UGGUGUAGCU  
 AAACACGUU GUUCACGG 50
- (2) INFORMATION FOR SEQ ID NO:202:  
 (1) SEQUENCE CHARACTERISTICS:

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- (A) LENGTH: 81 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:202:  
 AGAUCCUGU CGAGCAUCGUU UGGGUGGAG G  
 GCAGCTUGAG CUAACAGCU TUTGGACAG G
- (2) INFORMATION FOR SEQ ID NO:203:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 75 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:203:  
 AGAUCCUGU CGAGCAUCGUU GGAGGCGGAU CUAACAGCU GUAACAGCU  
 GUAGCCTUG AGCAGCU ACGG 50
- (2) INFORMATION FOR SEQ ID NO:204:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 74 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:204:  
 AGAUCCUGU CGAGCAUCGUU GUAACAGCU AUUGAAGAG AGTAAAGCCUG  
 UACCUACAGCU GCTTGUGGCA 50
- (2) INFORMATION FOR SEQ ID NO:205:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 76 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:205:  
 AGAUCCUGU CGAGCAUCGUU UGGGAGGUG UGGGCGGCA  
 CGUAGCUAA CACGTUGUC GACGG 50
- (2) INFORMATION FOR SEQ ID NO:206:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 75 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:206:  
 AGAUCCUGU CGAGCAUCGUU GUAACAGCU AUUGAAGAG AGTAAAGCCUG  
 GUAGCCTUG AGCAGCU ACGG 50
- (2) INFORMATION FOR SEQ ID NO:207:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 72 base pairs  
   (B) TYPE: nucleic acid
- (2) INFORMATION FOR SEQ ID NO:208:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 71 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:208:  
 AGAUCCUGU CGAGCAUCGUU GGAGGCGCUU GCUUACGUAG  
 CUAACAGCU UUUGUGGACG G 50
- (2) INFORMATION FOR SEQ ID NO:209:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 72 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:209:  
 AGAUCCUGU CGAGCAUCGUU GGAGGCGCUU UGGGCGCCU SCUUGACCUA  
 GCUUACAGCU UUUGUGGACG GG 50
- (2) INFORMATION FOR SEQ ID NO:210:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 72 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:210:  
 AGAUCCUGU CGAGCAUCGUU GGAGGCGCUU UGGGCGCCU GCUUACGUAG  
 GCUUACAGCU UUUGUGGACG GG 50
- (2) INFORMATION FOR SEQ ID NO:211:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 79 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:211:  
 GGGAGAUGC UGUGGACAU UGUGGAGAG GAAGUTAGUA Gccuuuugugu  
 GCUUACAGCU AAACAGCUU GUUGGACGG 50
- (2) INFORMATION FOR SEQ ID NO:212:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 79 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear

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		(xi) SEQUENCE DESCRIPTION: SEQ ID NO:212: GGAGAAGGCC TGTGAGGCAU GTTGCAUCGG GAAUCGAGAU AGUAGGCCGA	50	(2) INFORMATION FOR SEQ ID NO:218: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 30 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single
(2)		INFORMATION FOR SEQ ID NO:213: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 79 base pair (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	79	(2) INFORMATION FOR SEQ ID NO:218: (i) SEQUENCE DESCRIPTION: SEQ ID NO:218: ACTTACGGGG TAGTGTGCGG TTGGGGCTTA
(2)		INFORMATION FOR SEQ ID NO:214: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 75 base Pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	50	(2) INFORMATION FOR SEQ ID NO:219: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 30 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear
		(xi) SEQUENCE DESCRIPTION: SEQ ID NO:214: GAGAGAUGCC UGCGCAAC GAGGAGGCG CGUUCGGCGC CGUUCGCGAC GUUCGCAAC ACCUUCGCG ACAGG	50	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:219: ACACCCGGGG TAGGGTTAGGA TGGGGGTGTC
(2)		INFORMATION FOR SEQ ID NO:215: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 75 base Pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	75	(2) INFORMATION FOR SEQ ID NO:220: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 30 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear
		(xi) SEQUENCE DESCRIPTION: SEQ ID NO:215: AGATGCTCTT CCGAGCTGTG NNNNNNNNN NNNNNNNNN NNNNNNNNN GTAGCTTACAC TCTTGTGG ACAGG	50	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:220: GCAAGTGTGC TGGTGTGGG GTGAGATGGG
(2)		INFORMATION FOR SEQ ID NO:216: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 30 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	75	(2) INFORMATION FOR SEQ ID NO:221: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 30 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear
		(xi) SEQUENCE DESCRIPTION: SEQ ID NO:216: TCACTTAGGT AGGTGTTGCT GATGTCAGTG	30	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:221: GAGAATAGT AGGTGCT AGGTGTTGCT
(2)		INFORMATION FOR SEQ ID NO:217: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 30 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	30	(2) INFORMATION FOR SEQ ID NO:222: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 30 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear
		(xi) SEQUENCE DESCRIPTION: SEQ ID NO:217: GTCAGCTTACCC GGTTGAGGCA AGGTGGTGT	30	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:222: GAGTTGAGGG TAGGGGTGGG ATGGGGTAAAC
(2)		INFORMATION FOR SEQ ID NO:223: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 30 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	30	(2) INFORMATION FOR SEQ ID NO:223: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 30 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear
		(xi) SEQUENCE DESCRIPTION: SEQ ID NO:223: GTCAGCTTACCC GGTTGAGGCA AGGTGGTGT	30	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:223: GTCAGCTTACCC GGTTGAGGCA AGGTGGTGT

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- ATGTGTCACC GTGGTGGAGGA AGGATGTTGT  
 (2) INFORMATION FOR SEQ ID NO:224:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 30 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear  
 GTCGGTGCGG GGTGGGAAAT GGATGGCGTT  
 (2) INFORMATION FOR SEQ ID NO:225:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 30 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear  
 GGTGGCCGGA GTGGTGGCA GTAGGGTGG  
 (2) INFORMATION FOR SEQ ID NO:226:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 30 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear  
 GCGCCTACGCA GGCGTAGGTGT GGATGCAGCC  
 (2) INFORMATION FOR SEQ ID NO:227:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 31 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear  
 GTTGTGGAT AGGTAGTG TGCAATGATC T  
 (2) INFORMATION FOR SEQ ID NO:228:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 30 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear  
 GTCGGTGCGG GGTGGGAAAT GGATGGCGTT  
 (2) INFORMATION FOR SEQ ID NO:229:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 30 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
 GGTGATCGGT AGGGTTGGT GGCGTCAAT  
 (2) INFORMATION FOR SEQ ID NO:229:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 30 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
 GCGGCTACGCA GGCGTAGGTGT GGATGCAGCC  
 (2) INFORMATION FOR SEQ ID NO:230:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 30 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear  
 GCGTGGTGGCT CGGGGTAGTG GGGGTTGGT  
 (2) INFORMATION FOR SEQ ID NO:231:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 30 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear  
 GAAATCAGTT AAGGTGTTGA GGGCGAGGTG  
 (2) INFORMATION FOR SEQ ID NO:232:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 30 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear  
 TACTGCCTCG TGTGGAGGA GGTGGGGA  
 (2) INFORMATION FOR SEQ ID NO:233:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 30 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear  
 GCGTACTGCG CGGAGGAGC TGTGGAGCAC  
 (2) INFORMATION FOR SEQ ID NO:234:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 30 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear  
 GTGACTACTC TCACCTCTAT GGAGGCTCA  
 (2) INFORMATION FOR SEQ ID NO:235:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 30 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
 GCGGCTACGCA GGCGTAGGTGT GGATGCAGCC

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- (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:235:  
**CATACGCG** TAGTAGTGT TGGTGTTTT
- (2) INFORMATION FOR SEQ ID NO:235:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 30 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:236:  
**GTTTACGCT AGGTGTTGAT GGGCTACTTT**
- (2) INFORMATION FOR SEQ ID NO:237:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 30 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:237:  
**GCGTTTACG CTGGCTAGT GTGGGTGTA**
- (2) INFORMATION FOR SEQ ID NO:238:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 30 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:238:  
**GGGACTGCTA GGAACTGGT TGGAAGCCCA**
- (2) INFORMATION FOR SEQ ID NO:239:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 30 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:239:  
**GTGATGATGT AGGCTGAT AGGCTAGGT**
- (2) INFORMATION FOR SEQ ID NO:240:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 105 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:240:  
**AGATGCCCTG CGAACATCT ANNANNNNN NNNNNNNN NNNNNNNN NNNNNNNN**  
**NNNNNNNNN NNDDNNNN GTAGCTAAC TGCTTGTGCG ACGGG**
- (2) INFORMATION FOR SEQ ID NO:241:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 60 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:241:  
**GCAAAAGCGG GGAAAGTCGA GAGGTGAGCT GAGGTGTTGG GGATGTAAT**  
**CCCTGTGAC**
- (2) INFORMATION FOR SEQ ID NO:242:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 60 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:242:  
**GACCGGGCGAG GGAGGTGCA GCAGGATGG GTAGTGCTA GGCCTCTCAA**  
**CTCTGGATG**
- (2) INFORMATION FOR SEQ ID NO:243:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 58 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:243:  
**AGCTGTGTC GTGCCCGTG GTGAGGTTG ATGGCTGGT AGCTGATGCC**  
**CATGGCGA**
- (2) INFORMATION FOR SEQ ID NO:244:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 60 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:244:  
**CTGCGGGCTA GAGGAGCT GTAGGGGCG GTTGACTCG TACTCTCAGG**  
**GCCTGGCA**
- (2) INFORMATION FOR SEQ ID NO:245:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 60 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:245:  
**TGCTCTAGC TGCTAGTGT AGGATGCC GGAGTAGTG GGAGTGGG**  
**TGCGATGCG**
- (2) INFORMATION FOR SEQ ID NO:246:  
 (1) SEQUENCE CHARACTERISTICS:

-163-

- (A) LENGTH: 60 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

50

GGCGGCGTGT GIGTAGTC GCACGTGGT TGGCGCGAGA GCTCTAGAGT

60

GCATATGCG

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:245:

GCTCTAGAGT

(2)

INFORMATION FOR SEQ ID NO:247:

(1)

SEQUENCE CHARACTERISTICS:

(A)

LENGTH: 59 base pairs

(B)

TYPE: nucleic acid

(C)

STRANDEDNESS: single

(D)

TOPOLOGY: linear

(xi)

SEQUENCE DESCRIPTION: SEQ ID NO:247:

AGGCCCTGAA GCGCTTGTT GCGGGGGGT AGCTAGAGTC TGCATGATGC

50

TACCCCAAG

(2)

INFORMATION FOR SEQ ID NO:248:

(1)

SEQUENCE CHARACTERISTICS:

(A)

LENGTH: 60 base pairs

(B)

TYPE: nucleic acid

(C)

STRANDEDNESS: single

(D)

TOPOLOGY: linear

(xi)

SEQUENCE DESCRIPTION: SEQ ID NO:248:

CCGTCATCA ACCCTGGAC GCGTTGTC TGTGTGAGG GAGGATGGAC

50

CCGAGCTG

(2)

INFORMATION FOR SEQ ID NO:249:

(1)

SEQUENCE CHARACTERISTICS:

(A)

LENGTH: 60 base pairs

(B)

TYPE: nucleic acid

(C)

STRANDEDNESS: single

(D)

TOPOLOGY: linear

(xi)

SEQUENCE DESCRIPTION: SEQ ID NO:249:

TGCGTCTGAA

50

TGAGCTCGAA

(2)

INFORMATION FOR SEQ ID NO:250:

(1)

SEQUENCE CHARACTERISTICS:

(A)

LENGTH: 60 base pairs

(B)

TYPE: nucleic acid

(C)

STRANDEDNESS: single

(D)

TOPOLOGY: linear

(xi)

SEQUENCE DESCRIPTION: SEQ ID NO:250:

TGAGCTGG TGAGGGTAG GGTGGGGTC CTGAGCGTC CTGATCACGC

50

TGAGCTGG

(2)

INFORMATION FOR SEQ ID NO:251:

(1)

SEQUENCE CHARACTERISTICS:

(A)

LENGTH: 60 base pairs

(B)

TYPE: nucleic acid

(C)

STRANDEDNESS: single

(D)

TOPOLOGY: linear

(xi)

SEQUENCE DESCRIPTION: SEQ ID NO:255:

GGAGCTGG CGAGGGAG AGTGGGGT GGCGAGCTGGT AGGGCGGTT

50

CGCTGTGCA

(2)

INFORMATION FOR SEQ ID NO:256:

(1)

SEQUENCE CHARACTERISTICS:

(A)

LENGTH: 60 base pairs

(B)

TYPE: nucleic acid

(C)

STRANDEDNESS: single

(D)

TOPOLOGY: linear

(xi)

SEQUENCE DESCRIPTION: SEQ ID NO:256:

CTGAGCGTC

(2)

INFORMATION FOR SEQ ID NO:257:

(1)

SEQUENCE CHARACTERISTICS:

(A)

LENGTH: 60 base pairs

(B)

TYPE: nucleic acid

(C)

STRANDEDNESS: single

(D)

TOPOLOGY: linear

-164-

(C)

STRANDEDNESS: single

(D)

TOPOLOGY: linear

(xi)

SEQUENCE DESCRIPTION: SEQ ID NO:251:

GCGAGCTGCT CTTCTGCAA GTGTGCTGTT CGGGAGAGGG TAGCTCTGGA

50

TGATCCCGAA

(2)

INFORMATION FOR SEQ ID NO:252:

(1)

SEQUENCE CHARACTERISTICS:

(A)

LENGTH: 60 base pairs

(B)

TYPE: nucleic acid

(C)

STRANDEDNESS: single

(D)

TOPOLOGY: linear

(xi)

SEQUENCE DESCRIPTION: SEQ ID NO:252:

CTAACGCGTC GTAGGGGTG TTGGAGTGG TGACTCCCGC TGCGCGTGT

50

TGCTCGAGGG

(2)

INFORMATION FOR SEQ ID NO:253:

(1)

SEQUENCE CHARACTERISTICS:

(A)

LENGTH: 60 base pairs

(B)

TYPE: nucleic acid

(C)

STRANDEDNESS: single

(D)

TOPOLOGY: linear

(xi)

SEQUENCE DESCRIPTION: SEQ ID NO:253:

CTGGGGGTGG GACGGAGCT GTAGGGCTAG GTGGAGTGGT GAGGGCGGTT

50

GAGCCGAGGCA

(2)

INFORMATION FOR SEQ ID NO:254:

(1)

SEQUENCE CHARACTERISTICS:

(A)

LENGTH: 60 base pairs

(B)

TYPE: nucleic acid

(C)

STRANDEDNESS: single

(D)

TOPOLOGY: linear

(xi)

SEQUENCE DESCRIPTION: SEQ ID NO:254:

GCACTAGGAA GCAAGGGGGC CTAGGGTAGG TTGGATGATGAT GGGGGCGGAC

50

GTCGCGACTT

(2)

INFORMATION FOR SEQ ID NO:255:

(1)

SEQUENCE CHARACTERISTICS:

(A)

LENGTH: 60 base pairs

(B)

TYPE: nucleic acid

(C)

STRANDEDNESS: single

(D)

TOPOLOGY: linear

(xi)

SEQUENCE DESCRIPTION: SEQ ID NO:255:

GGAGCTGG CGAGGGAG AGTGGGGT GGCGAGCTGGT AGGGCGGTT

50

CGCTGTGCA

(2)

INFORMATION FOR SEQ ID NO:256:

(1)

SEQUENCE CHARACTERISTICS:

(A)

LENGTH: 60 base pairs

(B)

TYPE: nucleic acid

(C)

STRANDEDNESS: single

(D)

TOPOLOGY: linear

(xi)

SEQUENCE DESCRIPTION: SEQ ID NO:256:

CTGAGCGTC

(2)

INFORMATION FOR SEQ ID NO:257:

(1)

SEQUENCE CHARACTERISTICS:

(A)

LENGTH: 60 base pairs

(B)

TYPE: nucleic acid

(C)

STRANDEDNESS: single

(D)

TOPOLOGY: linear

(xi)

SEQUENCE DESCRIPTION: SEQ ID NO:257:

GAGGGCGGTT AGGGCGGTT

50

GGGGCGGTT

(2)



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-168-

## (2) INFORMATION FOR SEQ ID NO:267:

## (1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 60 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:267: GAGACCTTGG TAGGCTGT TGGCCCTGG TGGAGCTGT CGAAGGAGG

GAGATCTCGG

(2) INFORMATION FOR SEQ ID NO:268:

## (1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 60 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:268: GGAAACCGGG AGGCGCTGG GTGGAGGGG TGGCCGATG TGGTAGGAC

GGACTCGGAT

(2) INFORMATION FOR SEQ ID NO:269:

## (1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 60 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:269: TGTTCAGT TCGCGCAGC TGTAGGATC AGGATGCGA GCTCGAAGAT

GTCGCGCAC

(2) INFORMATION FOR SEQ ID NO:270:

## (1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 60 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:270: CGAACTTGC GAGGTCGG CTTAGCGCTG GTAGCGTGG TTAGGGGCC

TGACCGGCC

(2) INFORMATION FOR SEQ ID NO:271:

## (1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 60 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:271: TGCTTGCGGC TGTCGCGACG GCCTCGTGG GGAGCTGG GGCAATCGTGG

ATGGGCCG

(2) INFORMATION FOR SEQ ID NO:272:

## (1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 75 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:272: AGATCCCTGT CGAACGACTC ACCCGCTGG TAGGTAGGA Tgggtggatc

GTAAGCTTAC

(2) INFORMATION FOR SEQ ID NO:273:

## (1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 75 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:273: AGATCCCTGT CGAACGACTC GTGATAAGT Tgggtggatc GGGTACCGT

GTAGCTAAC

(2) INFORMATION FOR SEQ ID NO:274:

## (1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 75 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:274: AGATCCCTGT CGAACGACTC GGGCTAGCA GGGTAGGT GTGAGCTGCC

GTAGCTAAC

(2) INFORMATION FOR SEQ ID NO:275:

## (1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 75 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:275: AGATCCCTGT CGAACGACTC GTGTTGGT GGTGGGT GGTAGCGT

GTAGCTAAC

(2) INFORMATION FOR SEQ ID NO:276:

## (1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 75 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:276: AGATCCCTGT CGAACGACTC GGAGTGTG GGAGTGGT Tgggtggatc

GTAGCTAAC

(2) INFORMATION FOR SEQ ID NO:277:

## (1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 105 base pairs

(B) TYPE: nucleic acid



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- (2) INFORMATION FOR SEQ ID NO:288:
- (1) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 77 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:288:  
GGAGACUAG AUAAACGU CAACACAC AGCUAACCAA GCACTUGUGC  
CCUCUCCGACA UGAGGCCCG ACCGGC 50 77
- (2) INFORMATION FOR SEQ ID NO:289:
- (1) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 77 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:289:  
GGAGACUAG AUAAACGU CAACACAC AGCUAACCAA GCACTUGUGC  
UGUUCGACA UGAGGCCCG ACCGGC 50 77
- (2) INFORMATION FOR SEQ ID NO:290:
- (1) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 77 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:290:  
GGAGACUAG AUAAACGU CAACACAC AGCUAACCAA GCACTUGUGC  
CCUCUCCGACA UGAGGCCCG ACCGGC 50 77
- (2) INFORMATION FOR SEQ ID NO:291:
- (1) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 77 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:291:  
GGAGACUAG AUAAACGU CAACACAC AGCUAACCAA GCACTUGUGC  
CCUCUCCGACA UGAGGCCCG ACCGGC 50 77
- (2) INFORMATION FOR SEQ ID NO:292:
- (1) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 77 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:292:  
GGAGACUAG AUAAACGU CAACACAC AGCUAACCAA GCACTUGUGC  
CCUCUCCGACA UGAGGCCCG ACCGGC 50 77
- (2) INFORMATION FOR SEQ ID NO:293:
- (1) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 76 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:293:  
GGAGACUAG AUAAACGU CAACACAC AGCUAACCAA GCACTUGUGC  
GGUUCGACA UGAGGCCCG ACCGGC 50 76
- (2) INFORMATION FOR SEQ ID NO:294:
- (1) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 77 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:294:  
GGAGACUAG AUAAACGU CAACACAC AGCUAACCAA GCACTUGUGC  
CCUCUCCGACA UGAGGCCCG ACCGGC 50 77
- (2) INFORMATION FOR SEQ ID NO:295:
- (1) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 77 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:295:  
GGAGACUAG AUAAACGU CAACACAC AGCUAACCAA GCACTUGUGC  
GUUCUCCGACA UGAGGCCCG ACCGGC 50 77
- (2) INFORMATION FOR SEQ ID NO:296:
- (1) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 77 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:296:  
GGAGACUAG AUAAACGU CAACACAC AGCUAACCAA GCACTUGUGC  
AACTUCGACA UGAGGCCCG ACCGGC 50 77
- (2) INFORMATION FOR SEQ ID NO:297:
- (1) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 77 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:297:  
GGAGACUAG AUAAACGU CAACACAC AGCUAACCAA GCACTUGUGC  
CACTUCGACA UGAGGCCCG ACCGGC 50 77
- (2) INFORMATION FOR SEQ ID NO:298:
- (1) SEQUENCE CHARACTERISTICS:

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	(A) LENGTH: 77 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	(x1) SEQUENCE DESCRIPTION: SEQ ID NO:298: GGGAGCUAG AAUAAAGCU CAAACCUUG AGAACAGGC UGGAGCAAGA CACTUCGACA UGGGGCGCG AUCCGGC	50
(2)	INFORMATION FOR SEQ ID NO:299:		
(1)	SEQUENCE CHARACTERISTICS:		
(A)	LENGTH: 77 base pairs		
(B)	TYPE: nucleic acid		
(C)	STRANDEDNESS: single		
(D)	TOPOLOGY: linear		
(x1)	SEQUENCE DESCRIPTION: SEQ ID NO:299: GGGAGCTAG AAUAAAGCT CAAACGUACU AACGUACU GACAGAGCAC CACTUCGACA UGAGGCCGG AUCCGGC	50	
(2)	INFORMATION FOR SEQ ID NO:300:		
(1)	SEQUENCE CHARACTERISTICS:		
(A)	LENGTH: 77 base pairs		
(B)	TYPE: nucleic acid		
(C)	STRANDEDNESS: single		
(D)	TOPOLOGY: linear		
(x1)	SEQUENCE DESCRIPTION: SEQ ID NO:300: GGGAGCTAG AAUAAAGCT CAAACGUACU AACGUACU GUGCACTUGG GUGGUCCGACA UGAGGCCGG AUCCGGC	50	
(2)	INFORMATION FOR SEQ ID NO:301:		
(1)	SEQUENCE CHARACTERISTICS:		
(A)	LENGTH: 79 base pairs		
(B)	TYPE: nucleic acid		
(C)	STRANDEDNESS: single		
(D)	TOPOLOGY: linear		
(x1)	SEQUENCE DESCRIPTION: SEQ ID NO:301: GGGAGAUCC UGUGAGCAU GCGCUCUGG URAAGCAUG UCGAAGUAC AUGAGUAGCU AAACAGCUU GUQACOGG	50	
(2)	INFORMATION FOR SEQ ID NO:302:		
(1)	SEQUENCE CHARACTERISTICS:		
(A)	LENGTH: 79 base pairs		
(B)	TYPE: nucleic acid		
(C)	STRANDEDNESS: single		
(D)	TOPOLOGY: linear		
(x1)	SEQUENCE DESCRIPTION: SEQ ID NO:302: GGGAGAUCC UGUGAGCAU GCGCUCUGG URAAGCAUG UCGAAGUAC CAUAGUAGCU AAACAGCUU GUQACOGG	50	
(2)	INFORMATION FOR SEQ ID NO:303:		
(1)	SEQUENCE CHARACTERISTICS:		
(A)	LENGTH: 79 base pairs		
(B)	TYPE: nucleic acid		
(C)	STRANDEDNESS: single		
(D)	TOPOLOGY: linear		
(x1)	SEQUENCE DESCRIPTION: SEQ ID NO:303: GGGAGAUCC UGUGAGCAU GCGCUCUGG URAAGCAUG UCGAAGUAC CAUAGUAGCU AAACAGCUU GUQACOGG	50	
(2)	INFORMATION FOR SEQ ID NO:304:		
(1)	SEQUENCE CHARACTERISTICS:		
(A)	LENGTH: 79 base pairs		
(B)	TYPE: nucleic acid		
(C)	STRANDEDNESS: single		
(D)	TOPOLOGY: linear		
(x1)	SEQUENCE DESCRIPTION: SEQ ID NO:304: GGGAGAUCC UGUGAGCAU GCGCUCUGG URAAGCAUG UCGAAGUAC CGCGAGACU AAACAGCUU GUQACOGG	50	
(2)	INFORMATION FOR SEQ ID NO:305:		
(1)	SEQUENCE CHARACTERISTICS:		
(A)	LENGTH: 79 base pairs		
(B)	TYPE: nucleic acid		
(C)	STRANDEDNESS: single		
(D)	TOPOLOGY: linear		
(x1)	SEQUENCE DESCRIPTION: SEQ ID NO:305: GGGAGAUCC UGUGAGCAU GCGCUCUGG URAAGCAUG UCGAAGUAC CAACGAGACU AAACAGCUU GUQACOGG	50	
(2)	INFORMATION FOR SEQ ID NO:306:		
(1)	SEQUENCE CHARACTERISTICS:		
(A)	LENGTH: 79 base pairs		
(B)	TYPE: nucleic acid		
(C)	STRANDEDNESS: single		
(D)	TOPOLOGY: linear		
(x1)	SEQUENCE DESCRIPTION: SEQ ID NO:306: GGGAGAUCC UGUGAGCAU GCGCUCUGG URAAGCAUG UCGAAGUAC AAUAGUAGCT AACAGCUU GUQACOGG	50	
(2)	INFORMATION FOR SEQ ID NO:307:		
(1)	SEQUENCE CHARACTERISTICS:		
(A)	LENGTH: 79 base pairs		
(B)	TYPE: nucleic acid		
(C)	STRANDEDNESS: single		
(D)	TOPOLOGY: linear		
(x1)	SEQUENCE DESCRIPTION: SEQ ID NO:307: GGGAGAUCC UGUGAGCAU GCGCUCUGG URAAGCAUG UCGAAGUAC ACAUGUAGCU AAACAGCUU GUQACOGG	50	
(2)	INFORMATION FOR SEQ ID NO:308:		
(1)	SEQUENCE CHARACTERISTICS:		
(A)	LENGTH: 79 base pairs		
(B)	TYPE: nucleic acid		
(C)	STRANDEDNESS: single		
(D)	TOPOLOGY: linear		

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:308:

GGAGAUGUC UGUCCAGCAU GCUGGGGAA CGCUCUUCGAC GAGUGACCC  
GCAGCAGCU AAACAGCTTU GUCCACCG

## (2) INFORMATION FOR SEQ ID NO:309:

## (1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 79 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:309:

GGAGAUGUC UGUCCAGCAU GCUGGGGAA GUACGUTSG GUCAAGCACA  
CCUCGAGCU AAACAGCTTU GUCCACCG

## (2) INFORMATION FOR SEQ ID NO:310:

## (1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 79 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:310:

GGAGAUGUC UGUCCAGCAU GCUGGGGAA UACAGUTTG GCAAGCACC  
CAACGAGCU AAACAGCTTU GUCCACCG

## (2) INFORMATION FOR SEQ ID NO:311:

## (1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 79 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:311:

GGAGAUGUC UGUCCAGCAU GCUGGGGAC GCUGUGACA AGUGCACCG  
CUCGAGCU AAACAGCTTU GUCCACCG

## (2) INFORMATION FOR SEQ ID NO:312:

## (1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 79 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:312:

GGAGAUGUC UGUCCAGCAU GCUGGGAA ACCURACGCC AAGCUCACCU  
CAGCGAGCU AAACAGCTTU GUCCACCG

## (2) INFORMATION FOR SEQ ID NO:313:

## (1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 79 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:313:

GGAGAUGUC UGUCCAGCAU GCUGGGAA ACCURACGCC AAGCUCACCU  
CAGCGAGCU AAACAGCTTU GUCCACCG

## (2) INFORMATION FOR SEQ ID NO:314:

## (1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 79 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:314:

GGAGAUGUC UGUCCAGCAU GCUGGGAA ACCURACGCC AAGCUCACCU  
CAGCGAGCU AAACAGCTTU GUCCACCG

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AAGUGUAGCU AAACAGCTTU GUCCACCG

## (2) INFORMATION FOR SEQ ID NO:314:

## (1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 79 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:314:

GGAGAUGUC UGUCCAGCAU GCUGGGAA CCGAUUUGCA AGACACCG  
CCCGUAGCU AAACAGCTTU GUCCACCG

## (2) INFORMATION FOR SEQ ID NO:315:

## (1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 79 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:315:

GGAGAUGUC UGUCCAGCAU GCUGGGAA AGUCCACCU  
AUGGUAGCU AAACAGCTTU GUCCACCG

## (2) INFORMATION FOR SEQ ID NO:316:

## (1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 79 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:316:

GGAGAUGUC UGUCCAGCAU GCUGGGAA CCUGAGCU AGUGCACCG  
ACAUUAGCU AAACAGCTTU GUCCACCG

## (2) INFORMATION FOR SEQ ID NO:317:

## (1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 79 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:317:

GGAGAUGUC UGUCCAGCAU GCUGGGAA CGUUGACAA GUACACCCAG  
UUUGGUAAGCU AAACAGCTTU GUCCACCG

## (2) INFORMATION FOR SEQ ID NO:318:

## (1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 79 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:318:

GGAGAUGUC UGUCCAGCAU GCUGGGAA CGACCGUCU UCACAGAGUC  
ACCUUGACU AAACAGCTTU GUCCACCG

## (2) INFORMATION FOR SEQ ID NO:319:

## (1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 79 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:319:

GGAGAUGUC UGUCCAGCAU GCUGGGAA ACCUACACCU AAAGACACCC  
GGAGAUGUC UGUCCAGCAU GCUGGGAA ACCUACACCU AAAGACACCC

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-178-

- (A) LENGTH: 77 base Pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:319:  
 CCGACUCG AAUAAAACCU CAATGGGC UAAACCAC ACACUACGCC  
 UGUUUGACA UAGGCCCG ACCCGC
- (2) INFORMATION FOR SEQ ID NO:320:  
 (A) LENGTH: 66 base Pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:320:  
 CGAAGCAGTG GANNNNNNN NNNNNNNN NNNGNNNNN NGAGCTTAA  
 CGSCGUUGUC GACGG
- (2) INFORMATION FOR SEQ ID NO:321:  
 (1) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 21 base Pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:321:  
 ATCCGCTCA TTAGGGATCT
- (2) INFORMATION FOR SEQ ID NO:322:  
 (1) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 86 base Pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:322:  
 ATCCGCTCA TAGGAGAC TNNNNNNN NNNNNNNN NNNNNNNN  
 NNNNNNNN NACTTGAGCA AAATCACCG CAGGG
- (2) INFORMATION FOR SEQ ID NO:323:  
 (1) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 28 base Pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:323:  
 ATCCGCTCA TTAGGAGAC TNNNNNNN NNNNNNNN NNNNNNNN  
 NNNNNNNN NACTTGAGCA AAATCACCG CAGGG
- (2) INFORMATION FOR SEQ ID NO:324:  
 (1) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 25 base Pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:324:  
 CTACCTCTAGCA TCTGACTCTC  
 CTACCTCTAGCA TCTGACTCTC
- (2) INFORMATION FOR SEQ ID NO:325:  
 (1) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 81 base Pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:325:  
 CTACTCTAGAT CTGACTACCN NNNNNNNN NNNNNNNN NNNNNNNN  
 NNNNNNNNNN NNNNNNNNNN TCAGTATTC C
- (2) INFORMATION FOR SEQ ID NO:326:  
 (1) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 25 base Pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:326:  
 ATCGTATGAG ACTAATAGA GGNNA
- (2) INFORMATION FOR SEQ ID NO:327:  
 (1) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 15 base Pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:327:  
 GGAGAGACGA TGCG
- (2) INFORMATION FOR SEQ ID NO:328:  
 (1) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 61 base Pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:328:  
 GGAGAGACCA TGGCGNNNN NNNNNNNN NNNNNNNN NNNNNCAGAC  
 GGAGAGACCA TGGCGNNNN NNNNNNNN NNNNNNNN NNNNNCAGAC
- (2) INFORMATION FOR SEQ ID NO:329:  
 (1) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 20 base Pairs  
 (B) TYPE: nucleic acid



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## (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:339:  
 ATTCGGCTTGA TTACGGATAC TACTGGGGCT ATTCGAAATTAA TCCGCTAGTG  
 GCTGATCTA CACTTGAGCA AATTCAGCTG CAGGG

(2) INFORMATION FOR SEQ ID NO:340:

## (1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:340:  
 RGGGCGTNTGC AAAN

(2) INFORMATION FOR SEQ ID NO:341:

## (1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 35 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:341:  
 ATSCCAGGGC TTACGAAATC GGCGCGCA TCCC

(2) INFORMATION FOR SEQ ID NO:342:

## (1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 82 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:342:  
 CTACCTACGA TTGTGACTAGC AGGCGTTT AACATGAGAC TAGTACACT

(2) INFORMATION FOR SEQ ID NO:343:

## (1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 82 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:343:  
 CTACCTACGA TTGTGACTAGC AGGCGTTT AACATGAGAC TAGTACACT

(2) INFORMATION FOR SEQ ID NO:344:

## (1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 82 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:344:  
 CTACCTACGA TTGTGACTAGC AGGCGTTT AACATGAGAC TAGTACACT

(2) INFORMATION FOR SEQ ID NO:345:

## (1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 82 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:345:  
 CTACCTACGA TTGTGACTAGC AGGCGTTT AACATGAGAC TAGTACACT

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## (2) INFORMATION FOR SEQ ID NO:345:

(1) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 81 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:345:  
 CTACCTACGA TTGTGACTAGC AGGCGTTT AACATGAGAC TAGTACACT

(2) INFORMATION FOR SEQ ID NO:346:

## (1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 79 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:346:  
 CTACCTACGA TTGTGACTAGC GGGGCTGC TAAAGTCGAA ATGGACACGC

(2) INFORMATION FOR SEQ ID NO:347:

## (1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 62 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:347:  
 CTACCTACGA TTGTGACTAGC AGGCGTTT AACATGAGAC TAGTACACT

(2) INFORMATION FOR SEQ ID NO:348:

## (1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 79 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:348:  
 CTACCTACGA TTGTGACTAGC AGGCGTTT AACATGAGAC TAGTACACT

(2) INFORMATION FOR SEQ ID NO:349:

## (1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 76 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:349:  
 CTACCTACGA TTGTGACTAGC AGGCGTTT AACATGAGAC TAGTACACT

(2) INFORMATION FOR SEQ ID NO:349:

## (1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 76 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:349:  
 CTACCTACGA TTGTGACTAGC GGGGGGGG TTGGAAAT CGACATACT

(2) INFORMATION FOR SEQ ID NO:350:

## (1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 76 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:350:  
 CTACCTACGA TTGTGACTAGC AGGCGTTT AACATGAGAC TAGTACACT

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(A) LENGTH: 76 base Pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:351:  
**CTTACTAGGA TCAGTACGG AGGCGTTGT AACATGGAC TAGTACACT**  
**ATGCTAGCTT ACTCTCTATG APTTCC**

(2) INFORMATION FOR SEQ ID NO:351:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:351:  
**GCGGCCTT GYAAAN**

(2) INFORMATION FOR SEQ ID NO:352:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 61 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:352:  
**GGAGAGACCA TCGGGGGGC TTTCGAAGT TTGGTTAATC ACCTGCGAGC**  
**GACGACGGGG A**

(2) INFORMATION FOR SEQ ID NO:353:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 61 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:353:  
**GCGAGGACCA TCGGGGCTA TGTAAATTAC TGCTGTACTA CGCATCGAC**  
**GACGACGGGG A**

(2) INFORMATION FOR SEQ ID NO:354:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 62 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:354:  
**GGAGAGACCA TCGGGGGGC TTTCGAAGT TTGGTTAATC ACCTGCGAGC**  
**GACGACGGGG A**

(2) INFORMATION FOR SEQ ID NO:355:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 61 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:355:  
**GGAGAGACCA TCGGGGGGC TTTCGAAGT TTGGTTAATC ACCTGCGAGC**  
**GACGACGGGG A**

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(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:355:  
**GGAGAGACCA TCGGGGGCT CTGGRAGTG AAATCCCAC TACCGCGAC**  
**GACGACGGGG A**

(2) INFORMATION FOR SEQ ID NO:356:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 61 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:356:  
**GCGAGGACCA TCGGGGGGC TTTCGAAGT TTGGTTAATC ACCTGCGAGC**  
**GACGACGGGG A**

(2) INFORMATION FOR SEQ ID NO:358:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 61 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:358:  
**GGAGAGACCA TCGGGGGCT ATGCCAAATT TCCAAACTAC TCGATCAGAC**  
**GACGACGGGG A**

(2) INFORMATION FOR SEQ ID NO:359:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 61 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:359:  
**GGAGAGACCA TCGGGGGCA CTTAACGGGG CTTTGTAAATA CCCTGGAC**  
**GACGACGGGG A**

(2) INFORMATION FOR SEQ ID NO:360:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 61 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:360:  
**GGAGAGACCA TCGGGGGCA CTTAACGGGG CTTTGTAAATA CCCTGGAC**  
**GACGACGGGG A**



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		(C) STRANDEDNESS: single
		(D) TOPOLOGY: linear
		(xi) SEQUENCE DESCRIPTION: SEQ ID NO:371: ATCCGGCTGAA TTAGCGATAC TTAACACT CAACTGGCAA CCTCCCGAAG
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		ATCCGGCTGAA TTAGCGATAC TTAACACT CAACTGGCAA CCTCCCGAAG
(2)	INFORMATION FOR SEQ ID NO:372:	
(1)	SEQUENCE CHARACTERISTICS:	
(A)	LENGTH: 86 base pairs	
(B)	TYPE: nucleic acid	
(C)	STRANDEDNESS: single	
(D)	TOPOLOGY: linear	
(xi)	SEQUENCE DESCRIPTION: SEQ ID NO:372: GGCAGTTRACC CACTTGAGCA AATTCACCTG CAGGG	
50	86	
(2)	INFORMATION FOR SEQ ID NO:373:	
(1)	SEQUENCE CHARACTERISTICS:	
(A)	LENGTH: 85 base pairs	
(B)	TYPE: nucleic acid	
(C)	STRANDEDNESS: single	
(D)	TOPOLOGY: linear	
(xi)	SEQUENCE DESCRIPTION: SEQ ID NO:373: ATCCGGCTGAA TTAGCGATAC TGACCGAC TGATGCGTC GCCTCCGAT	
50	85	
(2)	INFORMATION FOR SEQ ID NO:374:	
(1)	SEQUENCE CHARACTERISTICS:	
(A)	LENGTH: 86 base pairs	
(B)	TYPE: nucleic acid	
(C)	STRANDEDNESS: single	
(D)	TOPOLOGY: linear	
(xi)	SEQUENCE DESCRIPTION: SEQ ID NO:374: ATCCGGCTGAA TTAGCGATAC TGCTCCGA CGGAGCTAG TCGACACAGC CCCATATGGA TACTTGAGCA AATTCACCTG CAGGG	
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(2)	INFORMATION FOR SEQ ID NO:375:	
(1)	SEQUENCE CHARACTERISTICS:	
(A)	LENGTH: 86 base pairs	
(B)	TYPE: nucleic acid	
(C)	STRANDEDNESS: single	
(D)	TOPOLOGY: linear	
(xi)	SEQUENCE DESCRIPTION: SEQ ID NO:375: ATCCGGCTGAA TTAGCGATAC TGACCGAC TGATGCGTC CCTCCCGATA GGCGATTTAC	
50	86	
(2)	INFORMATION FOR SEQ ID NO:376:	
(1)	SEQUENCE CHARACTERISTICS:	
(A)	LENGTH: 86 base pairs	
(B)	TYPE: nucleic acid	
(C)	STRANDEDNESS: single	
(D)	TOPOLOGY: linear	
(xi)	SEQUENCE DESCRIPTION: SEQ ID NO:376: CTACCTTACGA TTGACTTACG TGGAGCTGT CCTGGACAGT TTCTGAGAGT ASCTTACTCT CATGATTTTC C	
50	71	
(2)	INFORMATION FOR SEQ ID NO:377:	
(1)	SEQUENCE CHARACTERISTICS:	
(A)	LENGTH: 82 base pairs	
(B)	TYPE: nucleic acid	
(C)	STRANDEDNESS: single	
(D)	TOPOLOGY: linear	
(xi)	SEQUENCE DESCRIPTION: SEQ ID NO:377: CTACCTTACGA TTGACTTACG TGGAGCTGT CCTGGACAGT TTCTGAGAGC TCTCCACCAA TAGCTTACTC TCATGAAFTT CC	
50	82	
(2)	INFORMATION FOR SEQ ID NO:378:	
(1)	SEQUENCE CHARACTERISTICS:	
(A)	LENGTH: 82 base pairs	
(B)	TYPE: nucleic acid	
(C)	STRANDEDNESS: single	
(D)	TOPOLOGY: linear	
(xi)	SEQUENCE DESCRIPTION: SEQ ID NO:378: CTACCTTACGA TTGACTTACG TGGAGCTGT CCTGGACAGT TTCTGAGAGC TCTCCACCAA TAGCTTACTC TCATGAAFTT CC	
50	82	
(2)	INFORMATION FOR SEQ ID NO:381:	
(1)	SEQUENCE CHARACTERISTICS:	
(A)	LENGTH: 82 base pairs	
(B)	TYPE: nucleic acid	
(C)	STRANDEDNESS: single	
(D)	TOPOLOGY: linear	
(xi)	SEQUENCE DESCRIPTION: SEQ ID NO:381: CTACCTTACGA TTGACTTACG TGGAGCTGT CCTGGACAGT TTCTGAGAGC	
50	82	

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CGACGANGTA TAGCTTACTC TCATGTTAFTT CC

- (2) INFORMATION FOR SEQ ID NO:382:
- (1) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 82 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:382: CTAACTTAGA TGTGACTAGC ACAGGAGTT TTAAAGCCAC AGTGAAGCG

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GGGAGGAGGA TGCGGAGGA GCTTGTGGC CGGCCAGGG GCATTCAGAC

- (2) INFORMATION FOR SEQ ID NO:388:
- (1) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 61 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:388: GGGAGGAGGA TGCGGAGGA GCTTGTGGC CGGCCAGGG GCATTCAGAC

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61

GACGACGGG A

- (2) INFORMATION FOR SEQ ID NO:389:
- (1) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 61 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:389: GGGAGGAGGA TGCGGAGGA GTAGACGTTA GTCACTCTG CTCVCCAGAC

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61

GACGACGGG A

- (2) INFORMATION FOR SEQ ID NO:390:
- (1) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 22 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:390: CGAGGAR-YT TYARYGCRC RG

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CGAGGAG-CTT TTAGGCCAC AGTT

- (2) INFORMATION FOR SEQ ID NO:391:
- (1) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 25 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:391: CGAGGAG-CTT TTAGGCCAC AGTT

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CGAGGAG-CTT TTAGGCCAC AGTT

- (2) INFORMATION FOR SEQ ID NO:392:

(1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 85 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(2) INFORMATION FOR SEQ ID NO:383:

- (1) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:383: CTAACTCTAGA TGTGACTAGC TGAGGGTT CCTTGACAGT TTCTGAGATA

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(2) INFORMATION FOR SEQ ID NO:384:

- (1) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 61 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:384: GGGAGGAGCA TGCGGAGGA GCTTGTGGC CGGCCAGGG GCATTCAGAC

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61

GACGACGGG A

(2) INFORMATION FOR SEQ ID NO:385:

- (1) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 61 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:385: CGGAGGAGCA TGCGGAGGA GCTTGTGGC CGGCCAGGG GCATTCAGAC

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61

GACGACGGG A

(2) INFORMATION FOR SEQ ID NO:386:

- (1) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 61 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:386: CGGAGGAGCA TGCGGAGGA GCTTGTGGC CGGCCAGGG GCATTCAGAC

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61

GACGACGGG A

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-192-

-192-

	(D) TOPOLOGY: linear (xi) SEQUENCE DESCRIPTION: SEQ ID NO:392: ATCCGGCTGA TTAGGATAC TTAGGATC CGTCACCTCG ACCTACGSGTC CAGTGGAAAT ACTTGACCA AATCACCGC AGGG	50
(2)	INFORMATION FOR SEQ ID NO:393: (1) SEQUENCE CHARACTERISTICS: (A) LENGTH: 86 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single	85
(2)	INFORMATION FOR SEQ ID NO:393: (1) SEQUENCE CHARACTERISTICS: (A) LENGTH: 86 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single	50
(2)	INFORMATION FOR SEQ ID NO:394: (1) SEQUENCE CHARACTERISTICS: (A) LENGTH: 86 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (xi) SEQUENCE DESCRIPTION: SEQ ID NO:394: ATCCGGCTGA TTAGGATAC TACAGGCAA CCGGGTACAT AGTTTCGTT AAACTGACAC GACTGAGCA AATCACCG CAGGG	86
(2)	INFORMATION FOR SEQ ID NO:395: (1) SEQUENCE CHARACTERISTICS: (A) LENGTH: 86 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single	50
(2)	INFORMATION FOR SEQ ID NO:395: (1) SEQUENCE CHARACTERISTICS: (A) LENGTH: 86 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (xi) SEQUENCE DESCRIPTION: SEQ ID NO:395: ATCCGGCTGA TTAGGATAC TCYAGCTGT CGTCACCTCG CAGGG CACTAAACTC AATTCGAGCA AAATCACCG	86
(2)	INFORMATION FOR SEQ ID NO:396: (1) SEQUENCE CHARACTERISTICS: (A) LENGTH: 86 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (xi) SEQUENCE DESCRIPTION: SEQ ID NO:396: ATCCGGCTGA TTAGGATAC TCYAGCTGT CGTCACCTCG GITGAAAACC CACTAAACTC AATTCGAGCA AAATCACCG CAGGG	50
(2)	INFORMATION FOR SEQ ID NO:397: (1) SEQUENCE CHARACTERISTICS: (A) LENGTH: 86 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (xi) SEQUENCE DESCRIPTION: SEQ ID NO:397:	86
	(D) TOPOLOGY: linear (xi) SEQUENCE DESCRIPTION: SEQ ID NO:398: ATCCGGCTGA TTAGGATAC TCGAAGGCA CTTCAGCTCG TTAATAGTT CCTGCGACA TACTCGAGCA AATCACCGC CAGGG	50
(2)	INFORMATION FOR SEQ ID NO:399: (1) SEQUENCE CHARACTERISTICS: (A) LENGTH: 80 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (xi) SEQUENCE DESCRIPTION: SEQ ID NO:399: CTACCTTACCA TTCTGACTAC TACCCGCG TCGAGGTT CGCAGCCAC	86
(2)	INFORMATION FOR SEQ ID NO:400: (1) SEQUENCE CHARACTERISTICS: (A) LENGTH: 82 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (xi) SEQUENCE DESCRIPTION: SEQ ID NO:400: CTACCTTACCA TTCTGACTAC CTCAGTCAC GTCGGTCAC CTGGACTGAA AGTCAGTT TAGCTTACTC TCTATTAFTT CC	50
(2)	INFORMATION FOR SEQ ID NO:401: (1) SEQUENCE CHARACTERISTICS: (A) LENGTH: 82 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (xi) SEQUENCE DESCRIPTION: SEQ ID NO:401: CTACCTTACCA TTCTGACTAC CAACTCGCG AACACCCNGC AAGGTCCTC GGTCACTG TAGCTTACTC TCTATTAFTT CC	82
(2)	INFORMATION FOR SEQ ID NO:402: (1) SEQUENCE CHARACTERISTICS: (A) LENGTH: 63 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (xi) SEQUENCE DESCRIPTION: SEQ ID NO:402: CTACCTTACCA TTCTGACTAC ACTGCAACG GTTATGGGG CTAGCTACT CTCATGTTATC TCC	50
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- (2) INFORMATION FOR SEQ ID NO:403:
- (1) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 62 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:403:  
**CTTACCTAGA TCTGACTATGC ACTTGAGTAC CAGAAGTCCC TCGGCCCTG**  
**AATGGGCA A TACTTATC TGTGTTT CC**

50  
82

-194-

- (2) INFORMATION FOR SEQ ID NO:404:
- (1) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 61 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:404:  
**GCGAGGAGGA TGCGTCGC GGTATAGGC CTAGGTTG GTTACCA GAGC**  
**GAGACGGG A**

50  
61

-195-

- (2) INFORMATION FOR SEQ ID NO:405:
- (1) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 61 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:405:  
**GAGAGAGAGA TGAGGCTG GGGATTTC TTGGACTTC AGTACAGAC**  
**GAGGACGGG A**

50  
61

-196-

- (2) INFORMATION FOR SEQ ID NO:406:
- (1) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 61 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:406:  
**GGAGGAGGA TGGGGCGG ATTGGGGCA TAGGGCAC ACATAACAGAC**  
**GACGACGGG A**

50  
61

-197-

- (2) INFORMATION FOR SEQ ID NO:407:
- (1) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 60 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:407:  
**GGAGGAGGA TGCGGCGGC GACCGCGTA CAAGGATAG GGTACAGAC**  
**ACGACGGG A**

50  
60

-198-

- (2) INFORMATION FOR SEQ ID NO:408:
- (1) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 82 base pairs
  - (B) TYPE: nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:408:  
**CTTACCTAGA TCTGACTATGC AACGGATGA ACACCTACCA TCGAGGTGCC**  
**GCCTAAACG**

50  
86

-195-

-196-

		(C) STRANDEDNESS: single
		(D) TOPOLOGY: linear
	(xi)	SEQUENCE DESCRIPTION: SEQ ID NO:413: CTACCTTACAG TGTGACTAC ATGACGATC ATTAAGTCGG AAGATCTCG CAGTACGTT CC
(2)	INFORMATION FOR SEQ ID NO:414:	
(1)	SEQUENCE CHARACTERISTICS:	
(A)	LENGTH: 76 base pairs	
(B)	TYPE: nucleic acid	
(C)	STRANDEDNESS: single	
(D)	TOPOLOGY: linear	
(xi)	SEQUENCE DESCRIPTION: SEQ ID NO:414: CTACCTTACAG TGTGACTAC CACCTGCA GAGATCCGA CTCGGATGTT ATTGTTAGCTT ACTCTCTTG ARTTCC	50
(2)	INFORMATION FOR SEQ ID NO:415:	
(1)	SEQUENCE CHARACTERISTICS:	
(A)	LENGTH: 80 base pairs	
(B)	TYPE: nucleic acid	
(C)	STRANDEDNESS: single	
(D)	TOPOLOGY: linear	
(xi)	SEQUENCE DESCRIPTION: SEQ ID NO:415: CTACCTTACAG TTGTGACTAC CACCTGCA GAGATCCGA CTCGGATGTT ATCTTACCTA GCTTACTCTC ATGGTATTTCC	50
(2)	INFORMATION FOR SEQ ID NO:416:	
(1)	SEQUENCE CHARACTERISTICS:	
(A)	LENGTH: 61 base pairs	
(B)	TYPE: nucleic acid	
(C)	STRANDEDNESS: single	
(D)	TOPOLOGY: linear	
(xi)	SEQUENCE DESCRIPTION: SEQ ID NO:416: GGGAGGAGCA TGCGGAGGAC TGTGACCGCA CGGGTGTACAC TCTGGCAGAC GACGACOGCC A	50
(2)	INFORMATION FOR SEQ ID NO:417:	
(1)	SEQUENCE CHARACTERISTICS:	
(A)	LENGTH: 61 base pairs	
(B)	TYPE: nucleic acid	
(C)	STRANDEDNESS: single	
(D)	TOPOLOGY: linear	
(xi)	SEQUENCE DESCRIPTION: SEQ ID NO:417: GGAGGAGCA TGCGGACAC GGAGACCAAG GGAAATTCCA CGCGCAGAC GACGACOGCC A	50
(2)	INFORMATION FOR SEQ ID NO:418:	
(1)	SEQUENCE CHARACTERISTICS:	
(A)	LENGTH: 63 base pairs	
(B)	TYPE: nucleic acid	
(C)	STRANDEDNESS: single	
(D)	TOPOLOGY: linear	
(xi)	SEQUENCE DESCRIPTION: SEQ ID NO:418: ATCCGCGCTGA TTAGGATAC TACCCACAC CCCCTAAGAT TTAGAGCAA CTCGGCGCAA CACTGAGCA AAATCCTGT CAGGG	50
		61
		(xi) SEQUENCE DESCRIPTION: SEQ ID NO:418: GGGAGGAGCA TGCGGACAC TAGCGGAGG GAACTCTGAC AACATCGAC GACGACOGCC A
(2)	INFORMATION FOR SEQ ID NO:423:	
(1)	SEQUENCE CHARACTERISTICS:	
(A)	LENGTH: 86 base pairs	
(B)	TYPE: nucleic acid	
(C)	STRANDEDNESS: single	
(D)	TOPOLOGY: linear	
(xi)	SEQUENCE DESCRIPTION: SEQ ID NO:423: ATCCGCGCTGA TTAGGATAC TACCCACAC CCCCTAAGAT TTAGAGCAA CTCGGCGCAA CACTGAGCA AAATCCTGT CAGGG	50
		61

-197-

(2) INFORMATION FOR SEQ ID NO:424:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 88 base Pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:424:  
 ATCCGCTTCA TTACGGATC TGGAGACT GAGGCGTC CGCTCCGTT  
 CAGGTCACAT AGGACTTG AGAAATCC CGTCAGGG

(2) INFORMATION FOR SEQ ID NO:425:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 86 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:425:  
 ATCGCTGGA TAGCGATC TACGCCAG CCCTTAAGT TTAGAGCAA  
 CTCGCGCAA CACTGAGCA AAATCACCTG CAGGG

(2) INFORMATION FOR SEQ ID NO:426:

(1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 82 base Pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:426:  
 CTACCTTACGA TTGTACTAGC CACGGAGGT TGATTAAGGG TAGTCAAGG  
 TGCGGTATCC TAGCTTACTC TCTGTATT CC

(2) INFORMATION FOR SEQ ID NO:427:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 82 base Pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:427:  
 CTACCTTACGA TTGTACTAGC GACGGAGCA GTGCAAAAGG CTCATAGTAC  
 CGTCTAGTC TACTTACTC TCTGTATT CC

(2) INFORMATION FOR SEQ ID NO:428:

(1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 61 base Pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:428:  
 GGAGGAGGAGA TGCGGCCTCA GCGGATTCT TGGCAGTAG GAGCAGAC  
 GACGACCGGG A

(2) INFORMATION FOR SEQ ID NO:429:

(1) SEQUENCE CHARACTERISTICS:

-198-

(A) LENGTH: 61 base Pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:429:  
 GGGAGGAGCA TGCGGCAGC GACTTATA GTGTTATCC CGTACAGAC  
 GACGACCGGG A

-198-

(2) INFORMATION FOR SEQ ID NO:431:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 61 base Pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:431:  
 GGGAGGAGCA TGCGGCCTCA GCGGATTCT TGGCAGTAG GAGCAGAC  
 GACGACCGGG A

(2) INFORMATION FOR SEQ ID NO:432:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 87 base Pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:432:  
 ATCCGCTTCA TTACGGATC TGGAGACT GAGGCGTC CGCTCCGTT  
 GGTGGTCTCA GACTGAGC AAATCCCGT CGAGGG

(2) INFORMATION FOR SEQ ID NO:433:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 66 base Pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:433:  
 ATCCGCTTCA TTACGGATC TACAGTACCA TCCGGCCAG TGGTGGATT  
 TCAGAGTCTCA GACTGAGC AAATCCCGT CGAGGG

(2) INFORMATION FOR SEQ ID NO:434:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 82 base pairs  
   (B) TYPE: nucleic acid

(2) INFORMATION FOR SEQ ID NO:429:  
 (1) SEQUENCE CHARACTERISTICS:

-139-

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- (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear
- (xi)** SEQUENCE DESCRIPTION: SEQ ID NO: 434:  
**CTACTTACCA** TCTGACTAGC AGCTAGTCG A CTTCAGTAA CGCAGTGTT  
**GCGGAATCAG** TAGCTTACTC TCAATGATT CC 50  
**82**
- (2) INFORMATION FOR SEQ ID NO: 435:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 85 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear
- (xi)** SEQUENCE DESCRIPTION: SEQ ID NO: 435:  
**CTACTTACCA** TCTGACTAGC CCTCTAGT CGACCTGAG GCATGCAGC 60  
**TTCACCAT** GCGTAGCTA CTCCTAGTA TTTC 85
- (2) INFORMATION FOR SEQ ID NO: 436:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 62 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear
- (xi)** SEQUENCE DESCRIPTION: SEQ ID NO: 436:  
**GCGAGGACAC** TCGGGGCTT GTGACTGTGC TTATTCCTC CACATCAGAC  
**AGACGAGCGG** A 50  
**62**
- (2) INFORMATION FOR SEQ ID NO: 437:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 69 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear
- (xi)** SEQUENCE DESCRIPTION: SEQ ID NO: 437:  
**GCGAGGACAC** TCGGGGCTT GATGCGAGCT CGGAGGAGNA ANCCGAAAG  
**CCTACACAG** CAACGGGA 50  
**69**
- (2) INFORMATION FOR SEQ ID NO: 438:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 61 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear
- (xi)** SEQUENCE DESCRIPTION: SEQ ID NO: 438:  
**GCGAGGACAC** TCGGGGAGA GTCATCACCC GCACCACTC CTCTGAGAC  
**GACGACGGG** A 50  
**61**
- (2) INFORMATION FOR SEQ ID NO: 439:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 60 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear
- (xi)** SEQUENCE DESCRIPTION: SEQ ID NO: 439:  
**GCGAGGACAC** TCGGGGACTG GTCGCTGTC TTAGTCCCC CTCATCAGAC  
**GACGACGGG** A 50  
**61**
- (2) INFORMATION FOR SEQ ID NO: 439:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 60 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear
- (xi)** SEQUENCE DESCRIPTION: SEQ ID NO: 440:  
**GCGAGGACAC** TCGGGGAGC TTGCTGTC CACATCAGAC  
**GACGACGGG** A 50  
**61**
- (2) INFORMATION FOR SEQ ID NO: 441:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 61 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear
- (xi)** SEQUENCE DESCRIPTION: SEQ ID NO: 441:  
**GCGAGGACAC** TCGGGGACTC GATGCTCTA TGTCTGTCCT CTCGTCAGAC  
**GACGACGGG** A 50  
**61**
- (2) INFORMATION FOR SEQ ID NO: 442:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 61 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear
- (xi)** SEQUENCE DESCRIPTION: SEQ ID NO: 442:  
**GCGAGGACAC** TCGGGGAGA GTCATCACCC GCACCACTC CTCTGAGAC  
**GACGACGGG** A 50  
**61**
- (2) INFORMATION FOR SEQ ID NO: 443:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear
- (xi)** SEQUENCE DESCRIPTION: SEQ ID NO: 443:  
**GCGGGGCTA** CGTACGGG CTTTGAAA CCCGCC 37
- (2) INFORMATION FOR SEQ ID NO: 444:  
 (1) SEQUENCE CHARACTERISTICS:  
   (A) LENGTH: 26 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear
- (ix)** FEATURE:  
 (D) OTHER INFORMATION: All C's are 2'-NH<sub>2</sub> cytosine  
**(ix)** FEATURE:

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(1x) FEATURE: (D) OTHER INFORMATION: All U's are 2'-NH<sub>2</sub> uracil

(A) NAME/KEY: C

(B) LOCATION: 26

(D) OTHER INFORMATION: The C at location 26 is deoxyribose

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:444:  
GGGUGUUGGA AGACAGCGGG UGGUDC  
(2) INFORMATION FOR SEQ ID NO:445:  
(1) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 26 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear(xii) SEQUENCE DESCRIPTION: SEQ ID NO:445:  
CGTGTCGAGA AGACAGCGGG TGGTC  
26

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CLAIMS:

1. A method for identifying nucleic acid ligands to basic fibroblast growth factor (bFGF) comprising:
- preparing a candidate mixture of nucleic acids;
  - contacting the candidate mixture with bFGF, wherein nucleic acid ligands having an increased affinity to bFGF may be partitioned from the remainder of the candidate mixture;
  - partitioning between members of said candidate mixture on the basis of affinity to bFGF; and
  - amplifying selected molecules of the candidate mixture with a relatively higher affinity for bFGF to yield a mixture of nucleic acids enriched for sequences with a relatively higher affinity to the protein, whereby nucleic acid ligands of bFGF may be identified.
2. The method of claim 1 further comprising repeating steps b), c) and d).
3. The method of claim 1 wherein said candidate mixture of nucleic acids is comprised of single stranded nucleic acids.
4. The method of claim 3 wherein said candidate mixture of nucleic acids is comprised of RNA.
5. The method of claim 4 wherein said candidate mixture of nucleic acids is comprised of modified RNA.
6. The method of claim 5 wherein said candidate mixture of nucleic acids is comprised of RNA wherein all pyrimidines are 2'-deoxy-2'-NH<sub>2</sub> pyrimidines.
7. The method of claim 3 wherein said candidate

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mixture of nucleic acids is comprised of DNA.

forth in Tables II, III, IV and VIII.

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nucleic acid sequence of said ligand is substantially homologous to and has substantially the same ability to bind bFGF as a ligand selected from the group consisting of the sequences set forth in Tables II, III, IV and VIII.

- 8. A nucleic acid ligand to bFGF identified according to the method of claim 1.
- 5 9. The nucleic acid ligand of claim 8 comprising a single stranded nucleic acid.

- 10. The nucleic acid ligand of claim 8 comprised of RNA.

- 11. The nucleic acid ligand of claim 10 comprised of modified RNA.
- 15 12. The nucleic acid ligand of claim 11 comprised of RNA wherein all pyrimidines are 2'-deoxy-2'-NH<sub>2</sub> pyrimidines.

- 13. The nucleic acid ligand of claim 8 comprised of DNA.
- 20

- 14. The method of claim 2 further comprising f) identifying a nucleic acid ligand to bFGF from said mixture of nucleic acids enriched for sequences with a relatively higher affinity to bFGF.
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- 15. The method of claim 14 further comprising f) chemically modifying said identified nucleic acid ligand.
- 30

- 16. A purified and isolated non-naturally occurring RNA ligand to bFGF.
- 35

- 17. The RNA ligand of claim 16 wherein the nucleic acid sequence of said ligand is selected from the group consisting of the nucleotide sequences set

- 18. The RNA ligand of claim 16 wherein the nucleic acid sequence of said ligand is substantially homologous to and has substantially the same ability to bind bFGF as a ligand selected from the group consisting of the sequences set forth in Tables II, III, IV and VIII.

- 19. The RNA ligand of claim 16 wherein said ligand has substantially the same structure and substantially the same ability to bind bFGF as the sequences set forth in Tables II, III, IV, and VIII.

- 20. The RNA ligand of claim 16 wherein said ligand is an inhibitor of bFGF.

- 21. A purified and isolated non-naturally occurring DNA ligand to bFGF.
- 20

- 22. The DNA ligand of claim 21 wherein the nucleic acid sequence of said ligand is selected from the group consisting of the nucleotide sequences set forth in Tables XXI and XXII.
- 25

- 23. The DNA ligand of claim 21 wherein the nucleic acid sequence of said ligand is substantially homologous to and has substantially the same ability to bind bFGF as a ligand selected from the group consisting of the sequences set forth in Tables XXI and XXII.
- 30

- 24. The DNA ligand of claim 21 wherein said ligand has substantially the same structure and substantially the same ability to bind bFGF as the sequences set forth in Tables XXI and XXII.
- 35

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## 25. A method for treating bFGF-mediated

pathological conditions comprising administering a pharmaceutically effective amount of a nucleic acid bFGF ligand.

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26. The method of claim 25 wherein said nucleic acid bFGF ligand is identified according to the method of claim 1.

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27. The method of claim 25 wherein said ligand is selected from one of the 2'-NH<sub>2</sub>-modified ligands of Table VIII.

28. A method for identifying nucleic acid ligands to thrombin comprising:

a) preparing a candidate mixture of nucleic acids;

b) contacting the candidate mixture with thrombin, wherein nucleic acid ligands having an increased affinity to thrombin may be partitioned from the remainder of the candidate mixture;

c) partitioning between members of said candidate mixture on the basis of affinity to thrombin; and

d) amplifying selected molecules of the candidate mixture with a relatively higher affinity for thrombin to yield a mixture of nucleic acids enriched for sequences with a relatively higher affinity to the protein, whereby nucleic acid ligands of thrombin may be identified.

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31. The method of claim 30 wherein said candidate mixture of nucleic acids is comprised of RNA.

32. The method of claim 30 wherein said candidate mixture of nucleic acids is comprised of DNA.

33. A RNA nucleic acid ligand to thrombin identified according to the method of claim 28.

34. A DNA nucleic acid ligand to thrombin identified according to the method of claim 28.

35. The nucleic acid ligand of claim 32 being a single stranded nucleic acid.

36. A purified and isolated non-naturally occurring RNA ligand to thrombin wherein the nucleic acid sequence of said ligand is selected from the group consisting of the sequences set forth in Table XII.

37. The RNA ligand of claim 36 wherein said ligand is substantially homologous to and has substantially the same ability to bind thrombin as a ligand selected from the group consisting of the sequences set forth in Table XII.

38. The RNA ligand of claim 36 wherein said ligand has substantially the same structure and substantially the same ability to bind thrombin as the sequences set forth in Table XII.

39. A purified and isolated non-naturally occurring DNA ligand to thrombin wherein the nucleic acid sequence of said ligand is selected from the group consisting of the sequences set forth in Tables XV and XVI.

29. The method of claim 28 further comprising repeating steps b), c) and d).

30. The method of claim 28 wherein said candidate mixture of nucleic acids is comprised of single stranded nucleic acids.

35

35

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40. The DNA ligand of claim 39 wherein said ligand is substantially homologous to and has substantially the same ability to bind thrombin as a ligand selected from the group consisting of the sequences set forth in Table XV and XVI.

5  
10  
41. The DNA ligand of claim 39 wherein said ligand has substantially the same structure and substantially the same ability to bind thrombin as the sequences set forth in Table XV and XVI.

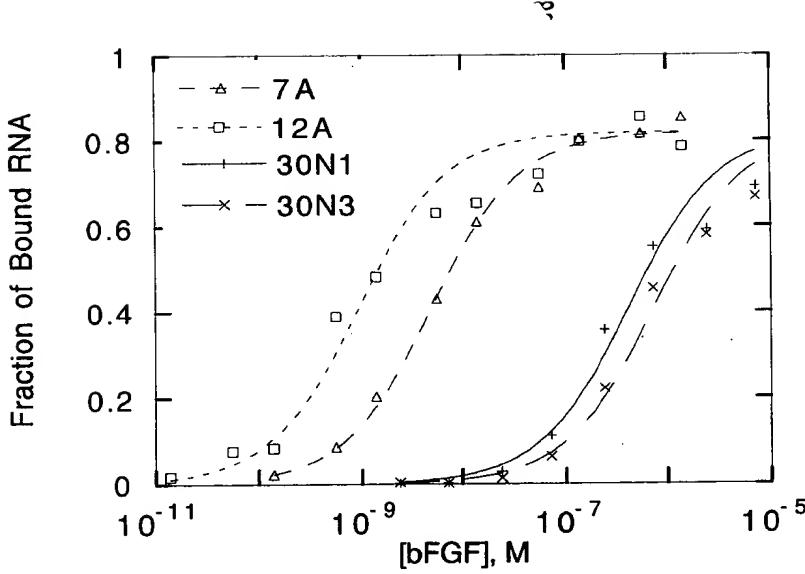
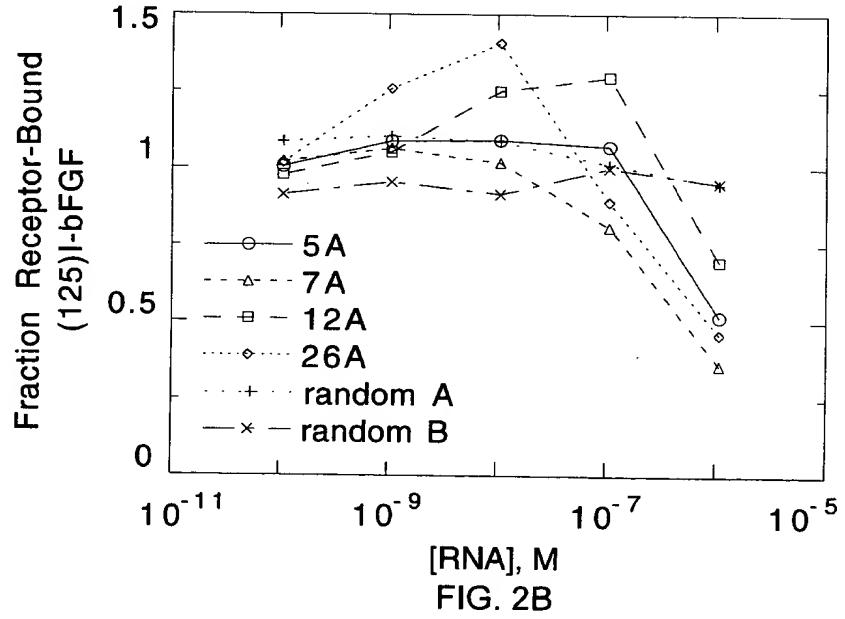
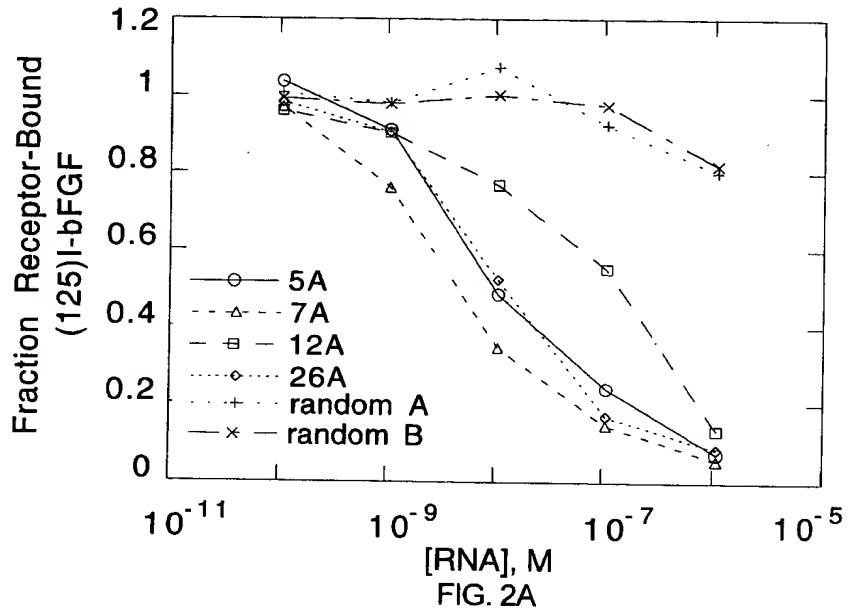


FIG. 1



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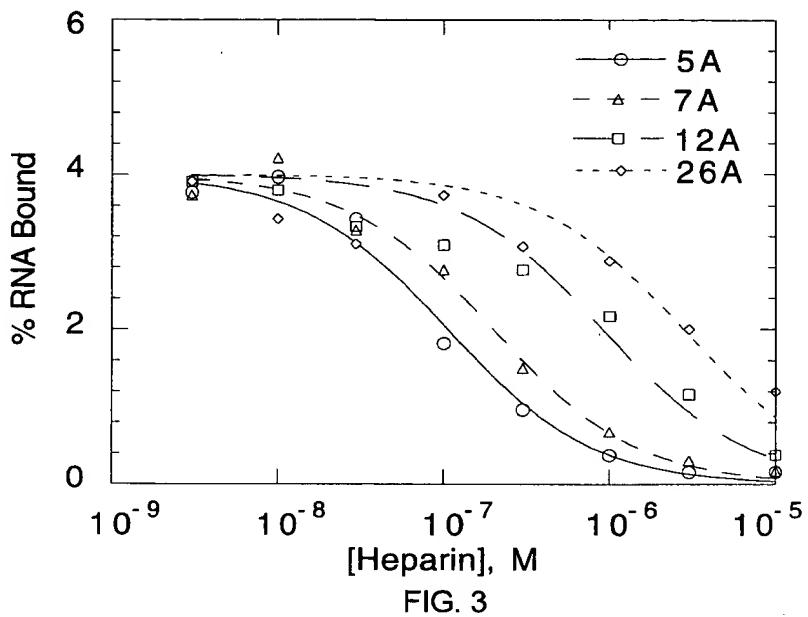


FIG. 3

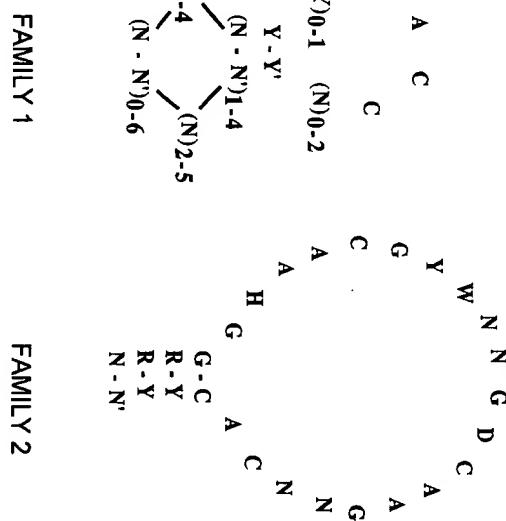


FIG. 4

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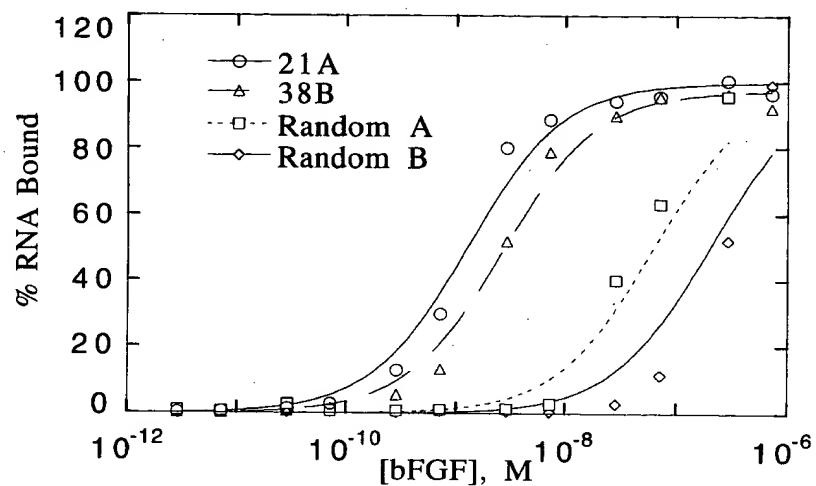


FIG. 5

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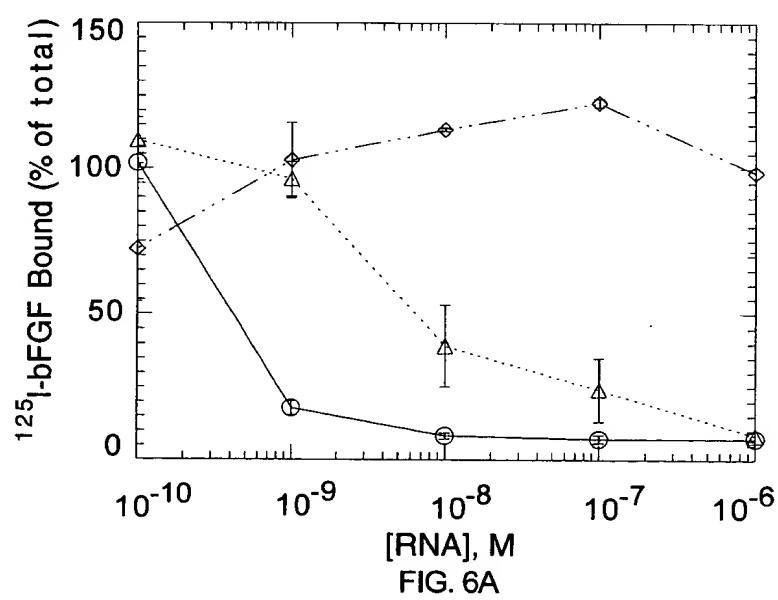


FIG. 6A

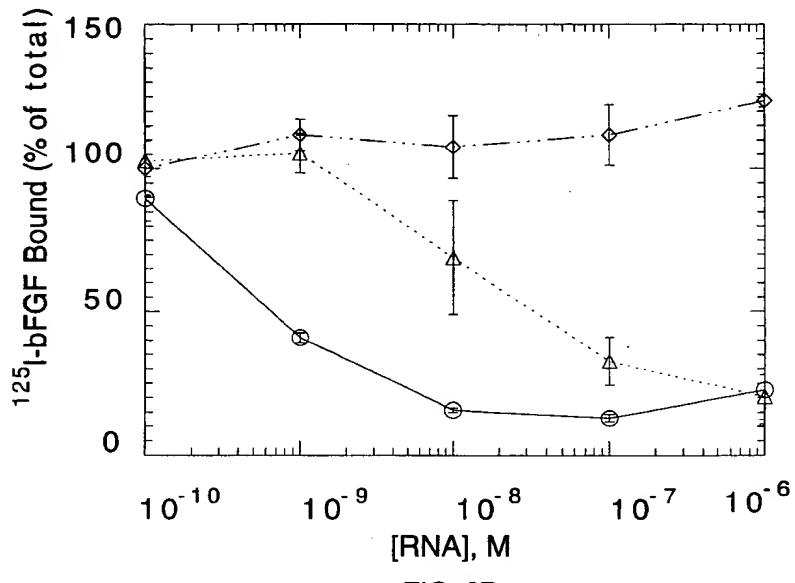


FIG. 6B

acgu = fixed region  
 ACGU = random region  
**ACGU** = conserved region  
 \_\_\_\_\_ = boundaries

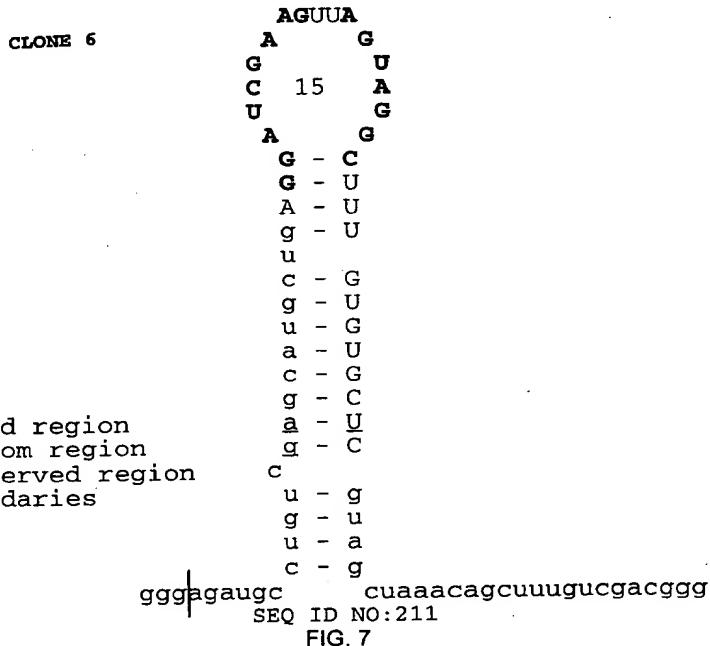
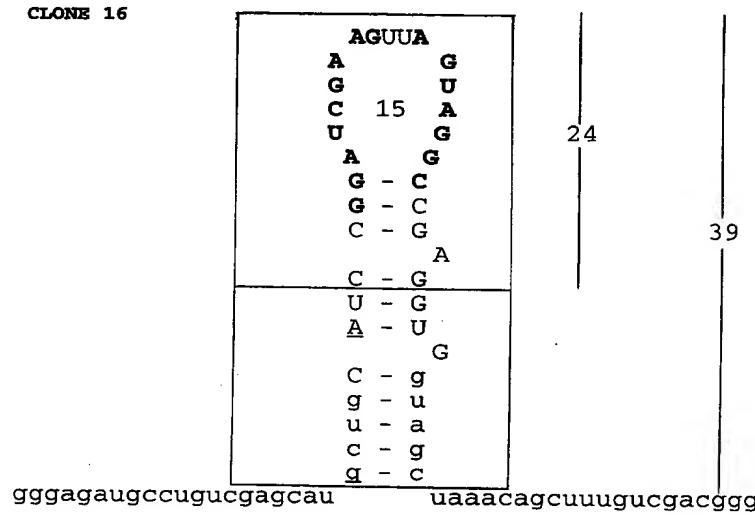


FIG. 7

## CLONE 16

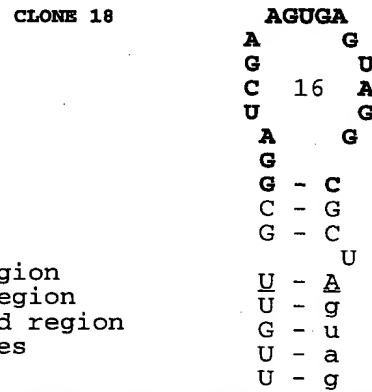


WO 09212601858

PCT/US95/01458

SEQ ID NO:212  
FIG. 7 (CONT'D)

## CLONE 18



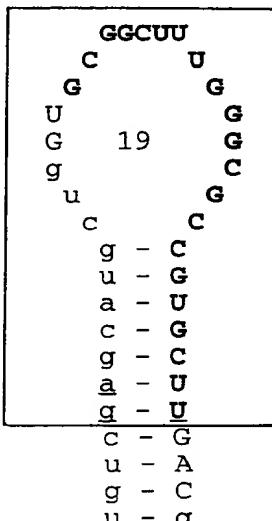
WO 09212601858

PCT/US95/01458

SEQ ID NO:213  
FIG. 7 (CONT'D)

acgu = fixed region  
 ACGU = random region  
**ACGU** = conserved region  
 \_\_\_\_\_ = boundaries

CLONE 27



acgu = fixed region  
 ACGU = random region  
**ACGU** = conserved region  
 \_\_\_\_\_ = boundaries

gggagaugcc uagcuaaagagcuuugucgacggg

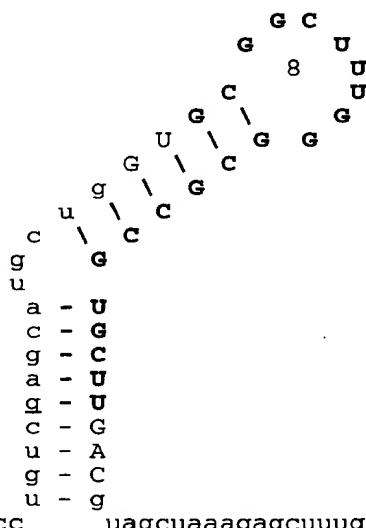
WO 95/21853

PCI/US93/01438

W0935

FCI/059/01438

SEQ ID NO: 214  
FIG. 7 (CONT'D)



acgu = fixed region  
ACGU = random region  
**ACGU** = conserved region  
= boundaries

gggagagaugcc uagcuaaagacguuugucgacggg

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SEQ ID NO: 215  
FIG. 7 (CONT'D)

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PCV1/83/50148

MO 9/21/83

PCV1/83/50148

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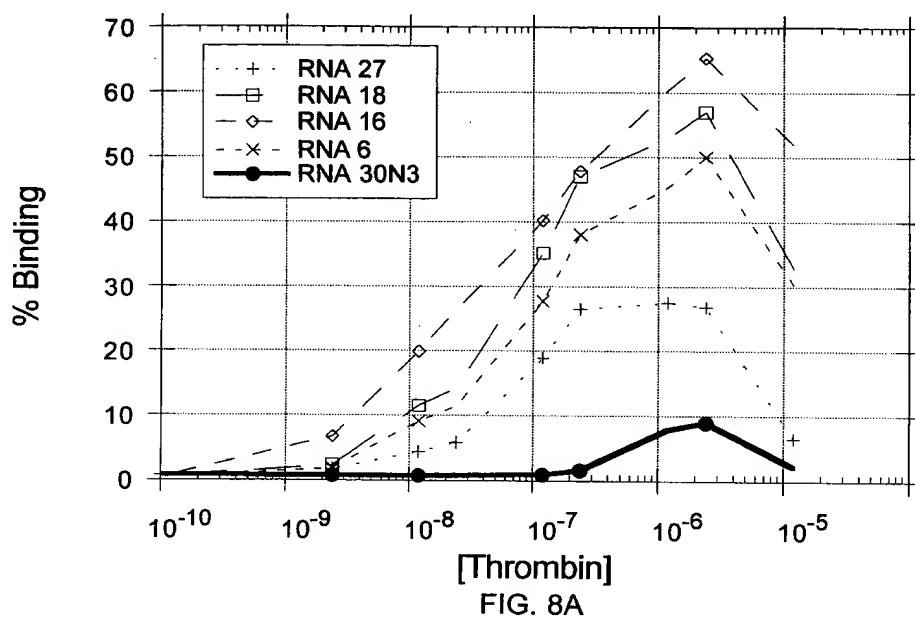


FIG. 8A

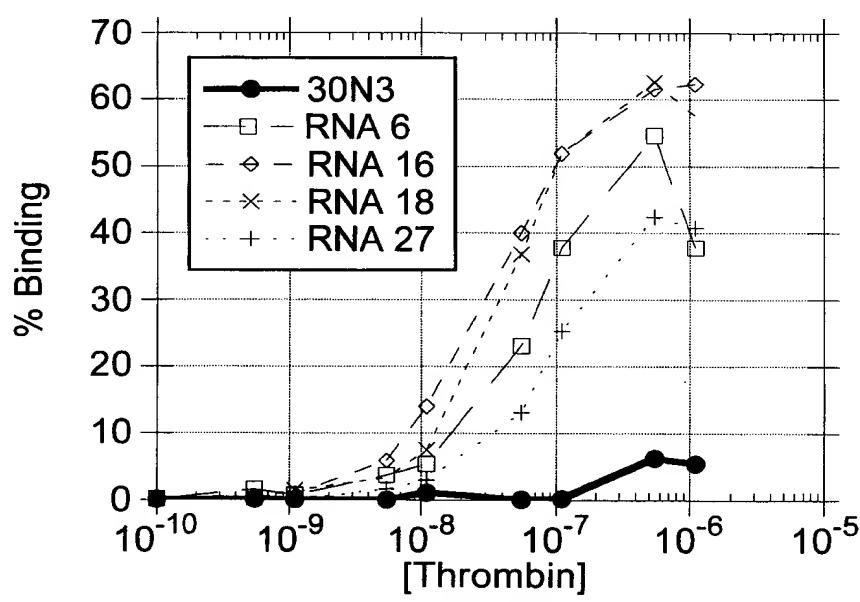


FIG. 8B

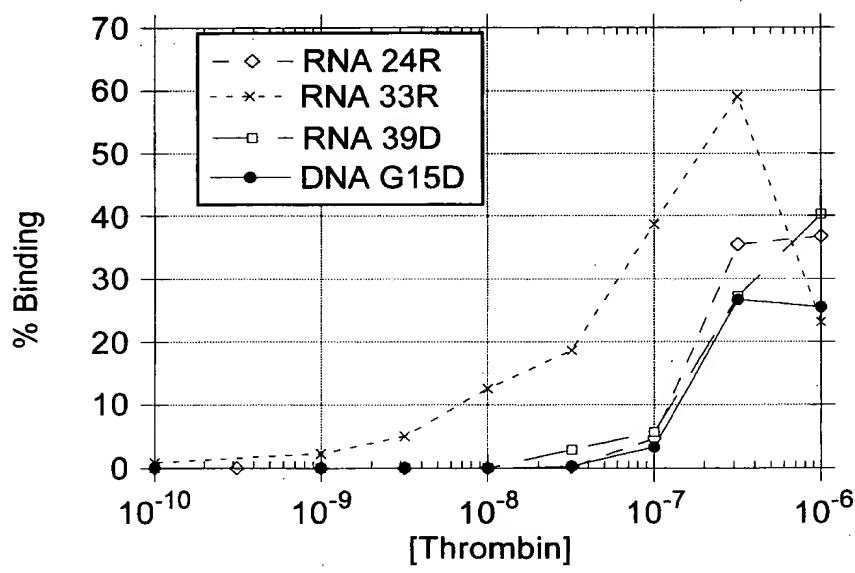


FIG. 8C

WO 952183

PC11550148

WO 952183

PC11550148

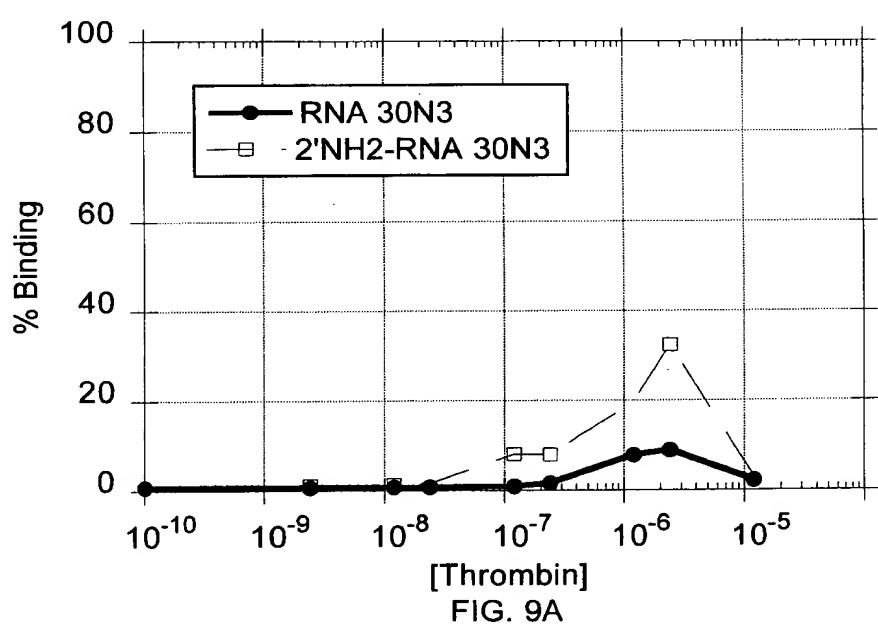


FIG. 9A

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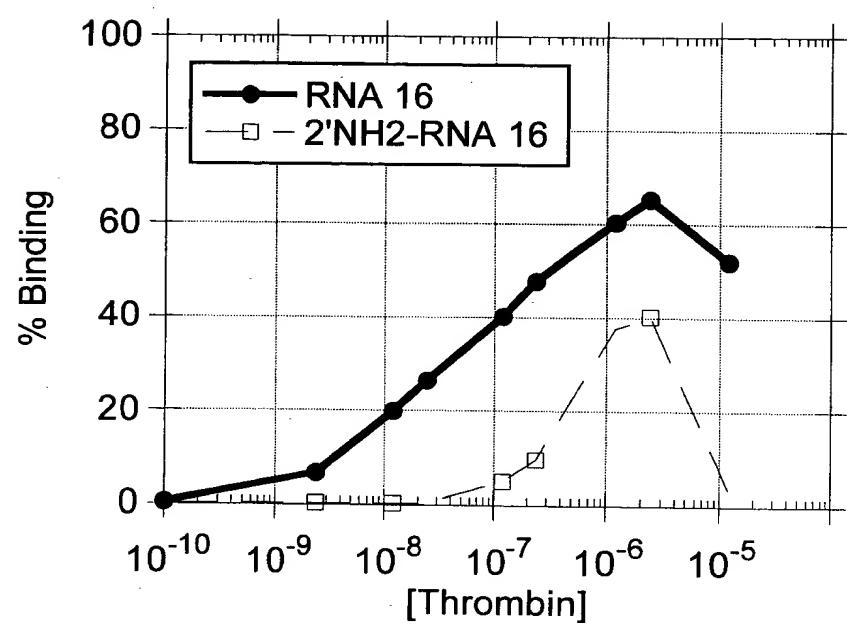


FIG. 9B

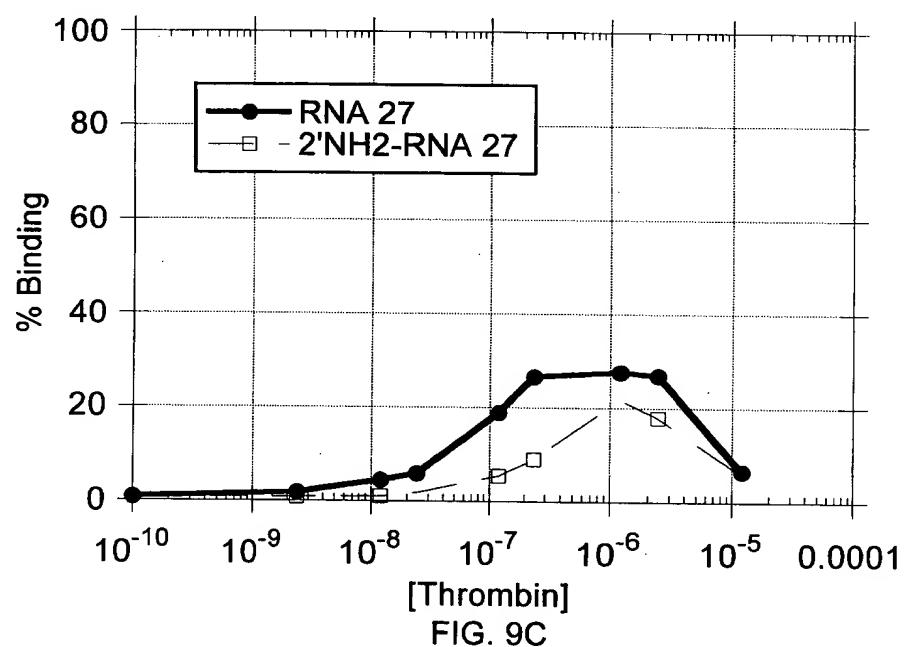


FIG. 9C

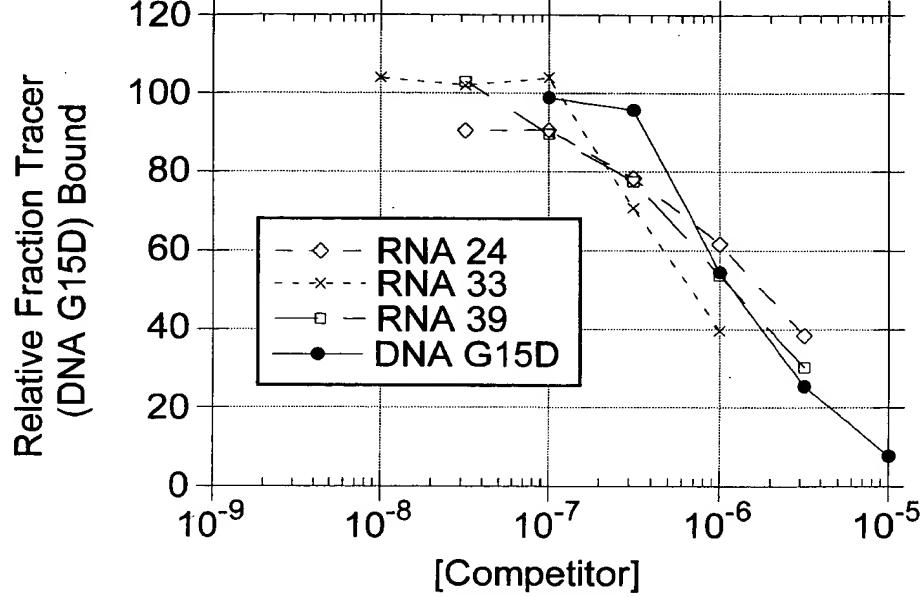


FIG. 10A

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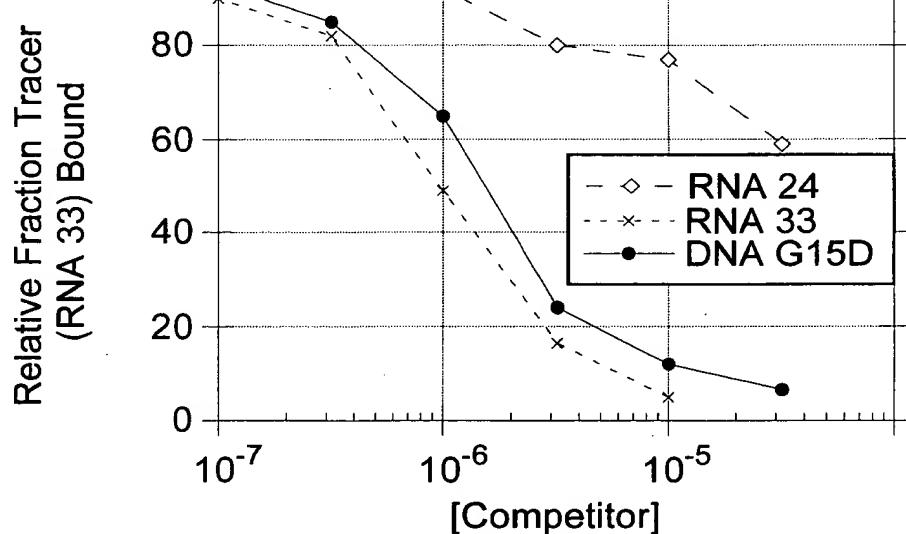


FIG. 10B

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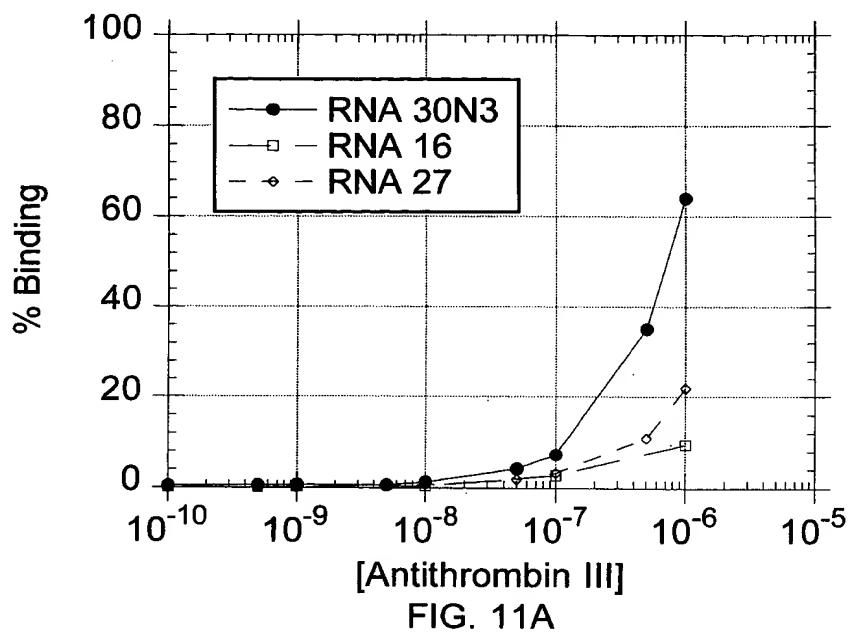


FIG. 11A

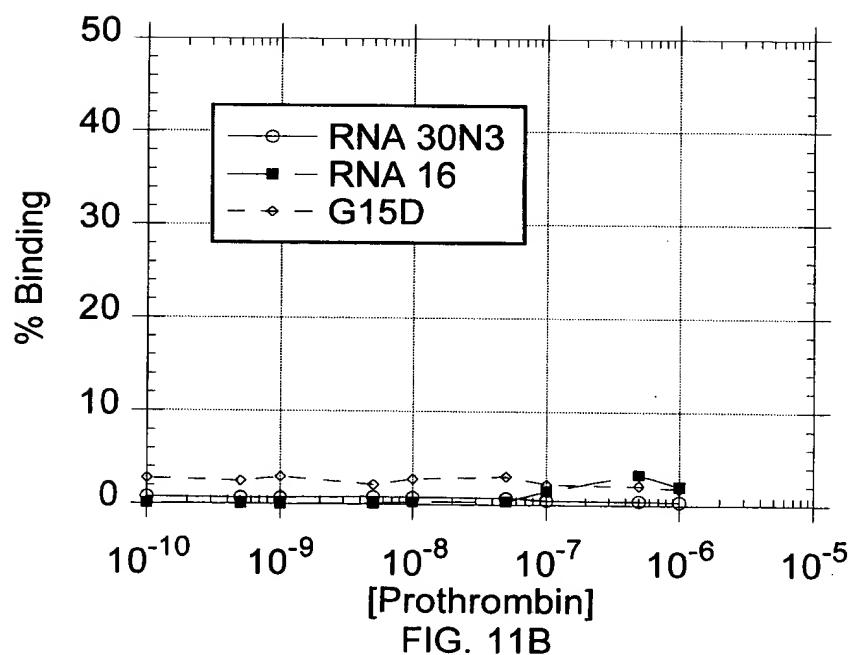
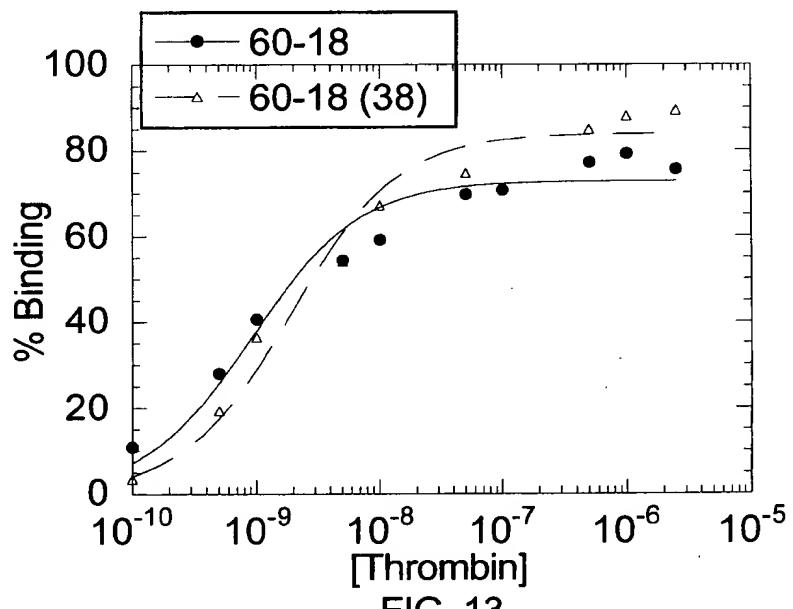
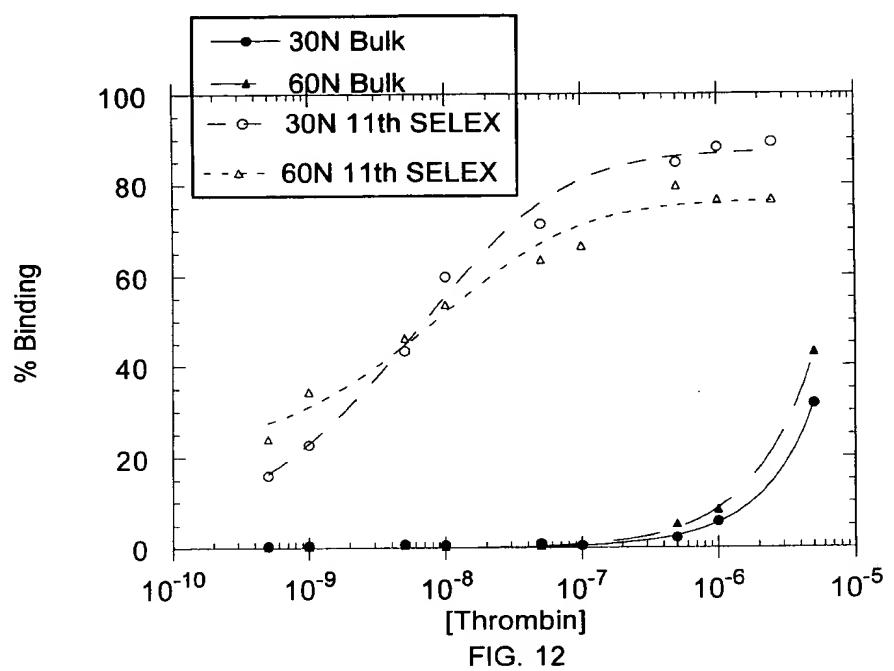


FIG. 11B



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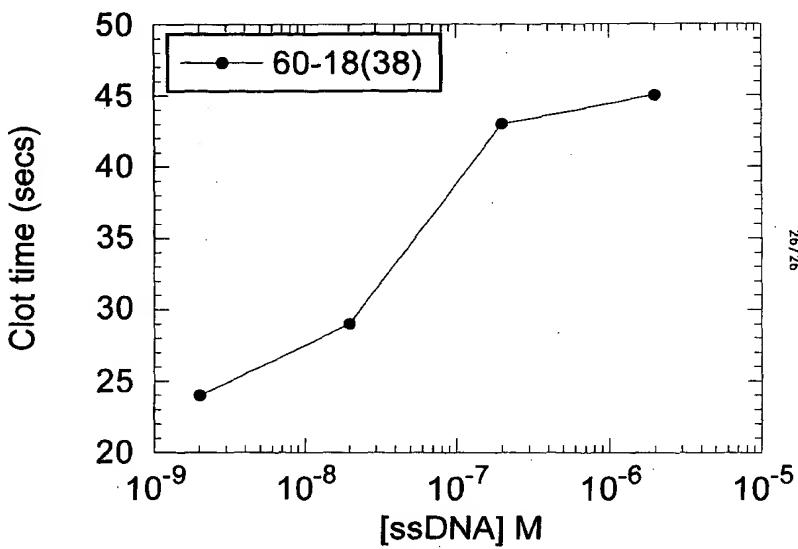


FIG. 14

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
APS, DIALOG: nucleic, binding, ligand, growth factor, thrombin

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Proceedings of the National Academy of Sciences, USA, Vol. 90, issued December 1993, Jellinek, D. et al., "High-Affinity RNA Ligands to Basic Fibroblast Growth Factor Inhibit Receptor Binding", pages 11227-11231, see entire document.	1-5, 8-10, 14, 16-20, 26 6, 7, 11-13, 15, 21-25
Y	Proceedings of the National Academy of Sciences, USA, Vol. 88, issued April 1991, Eriksson, A. et al., "Three-Dimensional Structure of Human Basic Fibroblast Growth Factor", pages 3441-3445, see entire document	1-27

D. Further documents are listed in the continuation of Box C.  See patent family chart.

* Special categories of cited documents:	Indicates whether the general name of the art which is not considered to be of particular relevance
TP	TP or TPI indicates that the document published earlier than the international filing date or priority date of the application, or later than the international filing date of the application, provides evidence of either the existence or non-existence of prior art which may affect the patentability of the claimed invention.
TJ	TJ indicates that the document published earlier than the international filing date or priority date of the application, or later than the international filing date of the application, provides evidence of either the existence or non-existence of prior art which may affect the patentability of the claimed invention.
TC	TC indicates that the document published earlier than the international filing date or priority date of the application, or later than the international filing date of the application, provides evidence of either the existence or non-existence of prior art which may affect the patentability of the claimed invention.
TO	TO indicates that the document published earlier than the international filing date or priority date of the application, or later than the international filing date of the application, provides evidence of either the existence or non-existence of prior art which may affect the patentability of the claimed invention.
TR	TR indicates that the document published prior to the international filing date but later than the international filing date of the application, provides evidence of either the existence or non-existence of prior art which may affect the patentability of the claimed invention.

Date of the actual compilation of the international search

10 MAY 1995

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## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US95/01458

C (Continuation), DOCUMENTS CONSIDERED TO BE RELEVANT	
Category*	Claim(s) of document, with indication, where appropriate, of the relevant passages
X	Nature, Vol. 355, issued 06 February 1992, Bock, L. et al., "Selection of Single-Stranded DNA Molecules That Bind and Inhibit Human Thrombin", pages 564-566, see entire document
Y	Science, Vol. 249, issued 03 August 1990, Tuerk, C. et al., "Systematic Evolution of Ligands by Exponential Enrichment: RNA Ligands to Bacteriophage T4 DNA Polymerase", pages 505-510, see entire document

Relevant to claim No.

X  
28-30, 32, 34,  
35,  
39-41  
31, 33, 36-38, 32

Y  
1-41  
1-27

